Jameel M. Al-Khayri · Shri Mohan Jain
Dennis V. Johnson *Editors*

Advances in Plant Breeding Strategies: Legumes

Volume 7

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Preface

Contemporary plant breeders no longer need to rely solely on traditional methodologies in their work of assuring a sustainable and elastic level of world food production. However, human population is increasing at an alarming rate in developing countries, and food availability could gradually become a serious problem. Agriculture production is severely affected because of environmental pollution, rapid industrialization, water scarcity and quality, erosion of fertile topsoil, limited remaining arable land to expand production area, lack of improvement of local plant types, erosion of genetic diversity, and dependence on only few crop species for food supply worldwide. According to FAO, 70% more food must be produced over the next four decades to feed a projected population of 9 billion people by the year 2050. Currently, only 30 plant species are used to meet 95% of the world's food requirements, which are considered as the *major crops.* The breeding programs of these crops have been very much dependent on the ready availability of genetic variation, either spontaneous or induced. Plant breeders and geneticists are under constant pressure to sustain and increase food production by using innovative breeding strategies and introducing minor crops that are well adapted to marginal lands and can provide source of nutrition through tolerance of abiotic and biotic stresses. In traditional breeding, introgression of one or a few genes into a cultivar is carried out via backcrossing over several plant life cycles.

With the development of new molecular tools, molecular marker-assisted backcrossing has facilitated rapid introgression of a transgene into a plant and reduced linkage drag. Continued development and adaptation of plant biotechnology, molecular markers, and genomics have established ingenious new tools for the creation, analysis, and manipulation of genetic variation for the development of improved cultivars. For example, molecular breeding has great potential to become standard practice in the improvement of several fruit crops. Adopting a multidisciplinary approach comprised of traditional plant breeding, mutation breeding, plant biotechnology, and molecular biology would be strategically ideal for developing new improved crop varieties. This book highlights the recent progress in the development of plant biotechnology, associated molecular tools, and their usage in plant breeding.

The basic concept of this book is to examine the best use of both innovative and traditional methods of plant breeding to develop new crop varieties suited to different environmental conditions to achieve sustainable food production, enhanced food security in a changing global climate as well as the development of crops for enhanced production of pharmaceuticals and innovative industrial uses. Three volumes of this book series were published in 2015, 2016, and 2018, respectively: Volume 1, *Breeding, Biotechnology and Molecular Tools;* Volume 2, *Agronomic, Abiotic, and Biotic Stress Traits*; and Volume 3, *Fruits*. In 2019, the following four volumes are concurrently being published: Volume 4, *Nut and Beverage* Crops; Volume 5, *Cereals*; Volume 6, *Industrial and Food* Crops; and Volume 7, *Legumes*.

This Volume 7, subtitled *Legumes,* focuses on advances in breeding strategies using both traditional and modern approaches for the improvement of individual legume crops. Included in this volume are adzuki bean, black gram, chickpea cluster bean, common bean, cowpea, faba bean, hyacinth bean, lentil, mung bean, pigeon pea, and soybean.

Chapters are written by internationally reputable scientists and subjected to a review process to assure quality presentation and scientific accuracy. Each chapter begins with an introduction covering related backgrounds and provides in-depth discussion of the subject supported with high-quality color photos, illustrations, and relevant data. This volume contains a total of 81 figures and 47 tables to illustrate presented concepts. The chapter concludes with an overview of the current status of breeding and recommendations for future research directions as well as appendixes listing research institutes and genetic resources relevant to the topic crop. A comprehensive list of pertinent references is provided to facilitate further reading.

The book is an excellent reference source for plant breeders and geneticists engaged in breeding programs involving biotechnology and molecular tools together with traditional breeding. It is suitable for both advanced undergraduate and postgraduate students specializing in agriculture, biotechnology, and molecular breeding as well as for seed companies and policy makers.

We are greatly appreciative of all chapter authors for their contributions toward the success and quality of this book. We are proud of this diverse collaborative undertaking, especially since this volume represents the efforts of 57 scientists from 9 countries. We are also grateful to Springer for giving us an opportunity to compile this book.

Al Hassa, Saudi Arabia — Jameel M. Al-Khayri
Helsinki, Finland — Shri Mohan Jain
Cincinnati, OH, USA — Dennis V. Johnson

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Jameel M. Al-Khayri is a Professor of Plant Biotechnology affiliated with the Department of Agricultural Biotechnology, King Faisal University, Saudi Arabia. He received his BS in Biology in 1984 from the University of Toledo, MS in Agronomy in 1988, and PhD in Plant Science in 1991 from the University of Arkansas. He is a member of the International Society for Horticultural Science and Society for In Vitro Biology as well as the National Correspondent of the International Association of Plant Tissue Culture and Biotechnology. His graduate work resulted in the establishment of in vitro regeneration protocols for spinach and zoysia grass. For the last two decades, he dedicated his research efforts to date palm. He has authored over 60 research articles in referred international journals and 25 review chapters and edited 7 journal special issues. In addition, he edited five reference books on date palm biotechnology and utilization of genetic resources and seven volumes of the book series advances in plant breeding strategies. He has been involved in organizing international scientific conferences and contributed numerous research presentations. In addition to teaching, students advising, and research, he held administrative responsibilities as the Assistant Director of Date Palm Research Center, Head of Department of Plant Biotechnology, and Vice Dean for Development and Quality Assurance. Dr. Al-Khayri served as a Member of Majlis Ash Shura

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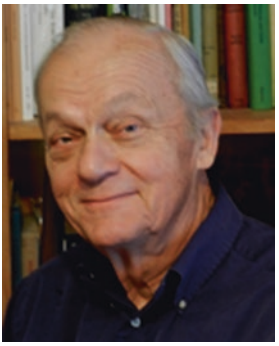
Shri Mohan Jain is a Consultant and Plant Biotechnologist, Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland; he received his MPhil in 1973 and PhD in 1978, Jawaharlal Nehru University, New Delhi, India. He was a Postdoctoral Fellow in Israel and the USA and Visiting Scientist/Professor in Japan, Malaysia, Germany, and Italy. He was a Technical Officer, Plant Breeding and Genetics, International Atomic Energy Agency (IAEA), Vienna, Austria, 1999–2005. He is a Member of the International Association of Plant Tissue Culture and Biotechnology and an Editorial Board Member of *Euphytica*, *In Vitro*, *Propagation of Ornamental Plants*, *Emirates Journal of Food and Agriculture*, and a series on *Forest Biotechnology*. His publications are more than 160 in peer-reviewed journals, book chapters, and conference proceedings, and he has edited 55 books and was invited speaker and acted as a Chairperson in several international conferences worldwide. He was awarded Nobel Peace Prize in 2005 in commemoration of the awarding to IAEA of the Nobel Peace Prize for 2005; he is also former consultant to IAEA, the European Union, the Government of Grenada, Iranian Private Company, and the Egyptian Government. Currently his research interests are somatic embryogenesis, organogenesis, haploidy, somatic cell hybridization, somaclonal variation, and mutagenesis mainly in medicinal plants, date palm, and banana genetic improvement, genetic diversity, erosion, conservation, and utilization in the context of climate change and food and nutritional security.



Dennis V. Johnson is a Consultant and former University Professor. He is a graduate of the University of California, Los Angeles, where he completed his BA (1966), MA (1970), and PhD (1972) degrees in Geography, with specialization in Agriculture and Biogeography. He has taught at several colleges and universities, including the University of Houston, and was a Visiting Professor for 2 years at the University of Ceará, Fortaleza, Brazil. Dr. Johnson also has worked extensively with international development agencies providing technical assistance to agriculture and forestry on projects and programs in Africa, Asia, Europe, and Latin America. He has published numerous articles on palm utilization and conservation and has edited or written books for FAO, IUCN, and UNEP. He has also translated into English plant science books from Portuguese and Spanish. A decade ago Dr. Johnson began to focus his research on date palm, in particular its introduction to nontraditional areas such as Spain, North and South America, and Australia. He co-authored a book on date growing in the USA and has made presentations at five international date palm conferences and co-edited books on date palm, sago palm, and plant breeding.

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Chapter 1
Adzuki Bean (*Vigna angularis* (Willd.) Ohwi & Ohashi) Breeding

Lixia Wang, Jie Wang, and Xuzhen Cheng

Abstract Adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi], an annual pulse crop, belongs to the genus *Vigna* and subgenus *Ceratotrapis*. It provides nutritional elements for the human diet and fertilizes soil by nitrogen fixation. It has been traditionally planted and consumed in East and Southeast Asia, especially in China, Japan and Korea, so it came to be called the *Asia legume*. Adzuki bean was dispersed to other continents for commercial uses in recent decades. Wild adzuki bean (*V. angularis* var. *nipponensis*), considered to be the ancestor of cultivated adzuki bean, occurs in East Asia and in the Himalayan Region, which are presumed to be where the domestication of adzuki bean took place. Another wild form, *V. nepalensis,* called the *weedy adzuki bean*, is mainly found in Eastern Nepal and around. A large portion of adzuki bean germplasm has been collected and conserved in different gene banks. DNA marker analysis suggests that there are obvious genetic distinctions between different forms, but the diversity among cultivated germplasm is quite low, indicating that the wild forms could be an important genetic resource for breeding. However, the genetic and genomic studies on this species are lagging and include only low-density genetic maps and a few maps of genes. That is the reason conventional breeding of adzuki bean has achieved rapid improvement, while no modern biotechnology has yet been used in breeding.

Keywords Biotechnology · Breeding · Conservation · Distribution · Diversity · Genetics

1.1 Introduction

Adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi], also called *azuki* or *aduki*, is an annual cultivated crop, belonging to the genus *Vigna* and subgenus *Ceratotropis*. Most of the collections, especially commercial varieties, have seeds with a uniform

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red color, so it is called *red bean* in folk parlance and is often confused with the red lucky seed tree (*Adenanthera pavonina*) which has poisonous red seeds. Adzuki bean is a diploid legume crop with 22 chromosomes (2n = 2x = 22) and an estimated genome size of about 500 Mb (Kang et al. 2015; Parida et al. 1990; Yang et al. 2015; Sakai et al. 2016), similar to its related crops, such as mung bean (*V. radiata*), rice bean (*V. umbellata*) and cowpea (*V. unguiculata*). The exact origin of adzuki bean is yet unknown; however, the presumed wild ancestor of cultivated adzuki bean is probably *V. angularis* var. *nipponensis*, which is distributed across Japan, Korea, China, Nepal, Bhutan and the Himalayan Region (Yamaguchi 1992). These regions, therefore, have a fairly high genetic diversity of adzuki bean. However, northeastern Asia is considered to be the initial domestication site of the crop according to archaeological findings (Lin et al. 2002). This chapter covers the distribution, production and uses of the adzuki bean, and also reports on the collection and conservation of germplasm resources, genetic studies and breeding, to provide an overview of the crop.

1.1.1 Distribution and Importance

As a warm-season pulse crop, adzuki bean is widely distributed in East and Southeast Asia, especially China, Japan and Korea, where it is usually called the *Asian legume* (Kramer et al. 2012; Tomooka et al. 2002). In recent decades, the cultivation of adzuki bean has spread to many countries in the world, such as Canada and Brazil, mainly for commercial uses. It is said the adzuki bean was recently planted in Africa as well, but there are no detailed statistical information available yet.

Compared with maize (*Zea mays*), wheat (*Triticum aestivum*), soybean (*Glycine max*) and other food crops, adzuki bean is a minor crop based on its planting area, total production and the consumption frequency in daily diets (Zheng 1997). However, it is certainly important, both in agricultural systems and modern dietary patterns. It can fertilize the soil by nitrogen fixation, and has become popular in intercropping and rotation systems. For instance, it is often intercropped with cotton and cereal crops in Northern China, or with sugarcane or young fruit trees in southern China, and grown in rotations in Japan.

Adzuki bean has been an important ingredient in desserts of East Asian diets and can be made into diverse sweetened bean paste. It also provides a source of plant protein for people from poor regions as a staple food. Adzuki bean is a traditional remedy too, especially in Chinese medicine, and popularly used in supplementary treatment of edema, eczema and other common diseases. Recent studies suggest that the functional factors, such as saponins and flavonoids in adzuki bean, ameliorate obesity by regulating lipid metabolism, as shown in animal tests (Kim et al. 2017; Liu et al. 2017a, b; Shi et al. 2017), which is providing an important basis to develop new health products.

1.1.2 Area and Production

Adzuki bean is temperature, moisture and light sensitive. The optimal temperature range for its growth is 25–35 °C. Excessively high temperatures will lead to slender seedlings, while low temperature will retard development. Germination needs soil temperatures above 6–10 °C (30–34 °C optimal). Adzuki bean can be sown in spring, summer or autumn, based on different climates, but it is always planted in summer in the northern and middle parts of China, where the main production areas in the country exist. Although adzuki bean can withstand drought to a certain degree (Luo et al. 2014) and it is usually not irrigated during the growth period, some rainfall is beneficial to boost the crop yield.

Over 20 countries plant adzuki bean at present; the largest are China, Japan and Korea, with estimated planting areas of 0.67, 0.12 and 0.03 million ha per year, respectively (Rubatzky et al. 1997). However, later estimates are lower, 0.3–0.4, 0.06–0.08 and 0.025 million ha, respectively (Cheng and Wang 2009). However, these data may be updated because in recent years, the annual area in China is currently estimated to be 0.45 million ha, and will increase with the recent policy changes of the agriculture structure at the national level. However, no recent official statistics are available.

Adzuki bean is the second most important legume after soybean in Japan. Hokkaido is the main production region, where full mechanization has been used in adzuki bean production leading to a relatively high economic efficiency. South Korea and other Southeast Asian countries also have areas of adzuki bean production, but the statistics of the total area are generally unknown.

Due to different cultivation intensity, ecological situations and mechanization level, the yield of adzuki bean varies considerably. A high yield of between 20–30 mt per ha was observed in Japan and North China, due to advanced breeding research and mechanization. However, the yield of this crop is much lower in most of countries or regions, and the production is mainly consumed by local people or for export under contract farming. It has been said that the annual world production of adzuki bean is about 800,000 mt (Vaughan et al. 2005), but no official statistics of world production of adzuki bean are available for recent years.

1.1.3 Nutritional Composition and Uses

The main component of adzuki bean is carbohydrates, accounting for about 40–60%, even reaching 65.5% (Orsi et al. 2017). Protein ranges from about 15–29% with an average of 23%, higher than any other major grain crops, and even animal products. Adzuki bean seed contains B vitamin folate, several mineral elements and negligible fat (Lin et al. 2002). Aside for the normal nutritional composition, functional factors have also been identified, such as saponins and general flavone (Liu et al. 2017a, b).

Traditionally, adzuki bean has been consumed as a food and is particularly popular in Asia countries. It is commonly sweetened before eating in East Asian cuisine. In particular, it is often boiled with sugar, resulting in red bean paste, a very common ingredient in all kinds of cuisines, including famous Chinese dishes, such as *tangyuan*, *mooncakes* and *baozi*, and the Japanese dishes, such as *anpan*, *dorayaki*, *imagawayaki*, *manjū*, *monaka*, *anmitsu*, *taiyaki* and *daifuku*. For the whole seeds, they can be consumed in diverse forms as well, such as mixed congee, ice candy, canned food, traditional Chinese rice pudding and Chinese bread, after being boiled. Puffed and fried food also use adzuki bean seeds in snack food in Southeast Asia. It is also traditionally cooked with rice in Japan and rural regions of North China, especially in scenic spots which attract tourists.

Adzuki bean is also a herbal medicine and traditionally used as a folk remedy in East Asia. For instance, it is usually used as an unguent by applying the juice and seed powder on the skin. The leaves are said to reduce fever and the sprouts are used to avert threatened abortion caused by injury. Adzuki bean is also used for the treatments of diverse diseases in Chinese folk medicine, such as kidney problems, constipation, abscesses, certain tumors and threatened miscarriage. Together with coarse cereals, adzuki bean is made into porridge for child-bearing women, according to the traditional idea that adzuki bean is a warm and blood-tonic food. With detailed research on the elements of adzuki bean, we believe that functional food to prevent or cure certain diseases can be developed in the near future (Baracho et al. 2016; Sato et al. 2016; Yao et al. 2015; Zhang and Wang 2016).

In addition to food and medicine, adzuki bean is also planted for fodder or green manure in regions of prosperous animal husbandry, and for soil conservation to ameliorate soil structure disorders.

1.1.4 Taxonomy and Biological Characteristics

Adzuki bean, a self-pollinating diploid legume with 2n = 22 chromosomes, together with mung bean, rice bean and black gram, belongs to the genus *Vigna*, subgenus *Cerototrapis* (Tomooka et al. 2002). Cultivated adzuki bean is a legume crop which has spread to many countries as mentioned above. Most of cultivated landraces are prostrate, but modern breeding cultivars are erect with a plant height 30–60 cm. Adzuki bean has two wild forms, wild and weedy. Wild adzuki bean has a wide distribution, including East Asia and Southeast Asia, especially the Himalayan Region (Tomooka et al. 2002). Wild forms are twinning herbs with slender stems and indeterminate growth habit. Weedy adzuki bean mainly occurs in Eastern Nepal and around, and in appearance is always between wild and cultivated types.

Adzuki bean seed takes at least a week to produce seedlings, slower than other *Vigna* crops. The emergence of the seedlings is hypogeal, different from mung bean or black gram. Usually it has classical pinnate compound leaf with three ovate or narrowly lobed leaflets, except for the first two simple and opposite leaves without petioles. Some germplasm has anthocyanidin coloration in the stem and petiole, making them appear somewhat purple, a trait which has been used as a character for

varieties. Papilionaceous flowers are golden or pale yellow, surrounded by 10 stamens and 1 pistil. The natural cross-pollination among adzuki bean is quite low. For cultivated adzuki bean, most of mature pods are yellow, yellow-white, brown or black with 4–12 seeds (Fig. 1.1). The seed weight varies from 5–20 g per 100 seeds (https://en.wikipedia.org/wiki/Adzuki_bean). Seed coats of wild forms are always brown, black or mottled, while for cultivated types, red or dark red seeds are common, and some accessions have green, yellow, black or dotted seeds (Fig. 1.2).

1.1.5 Domestication

Historical documents, carbonized seeds and starch granules from tombs provide evidence that China has a long history of adzuki bean planting, dating back about 2000 years (Zong et al. 2003a, b). Therefore, China is considered to be where the domestication of adzuki bean first took place (Lin et al. 2002). However, carbonized adzuki bean seeds found from archaeological sites in Japan suggest a planting history of over 3000 years (Yano et al. 2004). Based on recent genome sequence analysis, the genetic differentiation between wild and cultivated adzuki bean occurred

Fig. 1.1 Color of mature pods of cultivated adzuki bean. (Photo courtesy of Qingsheng Cai 2012)

Fig. 1.2 Color of seed coats of cultivated adzuki bean. (Photo courtesy of Lixia Wang 2017)

around 50,000 years ago (Kang et al. 2015), much earlier than what is estimated by archaeologists (Crawford 2006; Lee 2012), indicating a longer planting history of this crop. It is not yet known precisely where adzuki bean was first domesticated, although single or multiple domestication origins have been suggested (Kaga et al. 2008; Lee 2012; Yamaguchi 1992).

It is generally presumed that the wild ancestor of cultivated adzuki bean is *Vigna angularis* var. *nipponensis*, which is widely distributed in East Asia and Southeast Asia countries, including China, Japan, Korea, Nepal, Bhutan and the Himalayan Region (Kaga et al. 2008; Yamaguchi 1992). Another wild form of adzuki bean, *V. nepalensis*, only occurs in the southern foothills of the Himalayan mountainous regions (Tateishi and Maxted 2002), which has been called the adzuki bean complex, together with cultivated adzuki bean and *V. angularis* var. *nipponensis* (Tomooka et al. 2005). Except for cultivated and wild adzuki bean, there is a frequent and stable existence of an intermediate weedy form with an appearance between the wild and cultivated plants, mainly found in Japan (Tomooka et al. 2002; Vaughan et al. 2005), and which is a classical character for the origin place of the species. With the release of the whole genome sequences (Kang et al. 2015; Yang et al. 2015; Sakai et al. 2016) and the following extensive research on the different forms of adzuki bean, more information on the origin or domestication of this crop may be expected.

1.2 Conservation and Genetic Diversity

1.2.1 Germplasm Collection and Conservation

Since the end of the last century, many countries have shown interest in collecting adzuki bean germplasm to evaluate its diversity, and for the establishment of core collections (Frankel and Brown 1984). Efforts have been made on the germplasm collection of adzuki bean, including wild forms. Their seeds are conserved in different gene banks (Table 1.1).

Table 1.1 the main gene banks conserving adzuki bean collections

Genebank	Country	Number of accessions
Institute of Crop Sciences, CAAS	China	5500 (a few wild forms)
Hokkaido Prefectural Agricultural Experiment Station	Japan	3600
Genetic Resources Division, Rural Development Administration	Korea	3200 (a few wild forms)
Genetic Resources Center, National Agriculture and Food Research Organization	Japan	1900 (some wild forms)
National Plant Germplasm System	USA	660
Australia Plant Genetic Resources System		400
Asian Vegetable Research and Development Center	Thailand	150

Over 5000 collections are held in China; 98% are landraces collected within the country, while 2% were introduced from other countries (Hu et al. 1996; Zheng 1987, 1990). In recent years, wild adzuki bean has been observed and collected in several Chinese provinces, but extensive evaluations have not yet been performed. Korea ranks second in conserving over 3000 accessions and some of them have had detailed phenotypic evaluation (Yoon et al. 2012), while the scientists at the Asian Vegetable Research and Development Center (AVRDC) and in Japan have also collected a large number of accessions. The largest number of wild and weedy adzuki bean is conserved in Japan as well, and the genetic differentiation or divergence among them investigated by assessment of their hybrid affinity and comparative mapping (Kaga et al. 2008). In addition, there are some adzuki beans conserved in Europe and the Americas. These are derived from collecting expeditions in Asian countries or from introductions. For example, about 660 accessions of adzuki bean are conserved in the USA, most of them introduced from Korea, Japan and China (https://www.ars-grin.gov/npgs/aboutgrin.html#).

In situ conservation is complementary to ex situ conservation, and has been used in many species, especially for wild forms (N'Danikou et al. 2015). However, people are less aware of taking actions to protect adzuki bean or its wild forms in situ, mainly because adzuki bean is a fairly minor crop species that can easily set seeds (Tomooka et al. 2002). Because in situ conservation allows for continued evolution of genetic resources in their natural environment, and be helpful for genetic studies of evolution and new gene mining (Takahashi et al. 2016), it should be taken seriously where wild adzuki beans occur at a high density or exhibit high diversity.

1.2.2 Genetic Diversity and Characterization

Investigation of genetic differentiations among different forms of adzuki bean germplasm will be helpful in understanding the origin of adzuki bean, and to design breeding programs. Previous studies showed that both wild and weedy forms have higher genetic variations than cultivated forms (Mimura et al. 2000; Wang et al. 2011; Yoon et al. 2007) and adzuki bean in the Himalayan Region has a higher diversity than elsewhere (Zong et al. 2003b). The weedy form is a special genotype, which can be more easily used in breeding programs than the wild forms (Xu et al. 2000). DNA molecular markers also revealed that there is a 1% natural hybridization between wild and cultivated adzuki beans and the gene flow is stably inheritable (Yamamoto et al. 2006).

Distinct genetic distance between cultivated adzuki bean from different regions have been observed, indicating that cultivated adzuki bean is derived from different ancestors, and somewhat supports the theory that cultivated adzuki bean has multiple origins. In addition, adzuki beans from China, Japan and Korea, have closer genetic backgrounds than with those from the Himalayan Region, indicating adzuki bean from these three countries may have evolved from common ancestors (Zong et al. 2003a, b) (Fig. 1.3). Translocation of chromosomes in wild genotypes from a

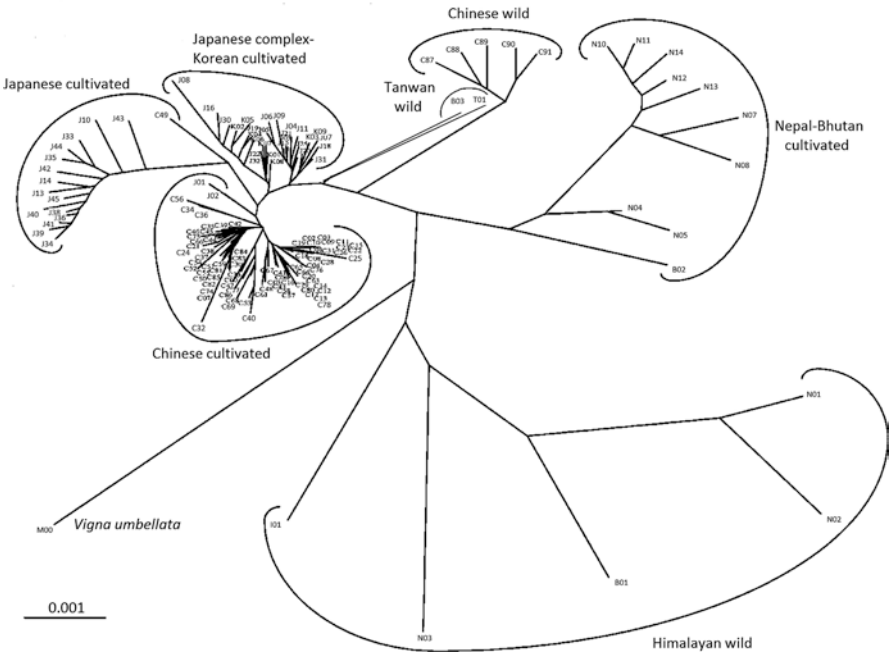

Fig. 1.3 A neighbor-joining tree based on pairwise distance using Innan's uncleotide diversity data (showing evolutionary groups). (Source: Zong et al. 2003a)

certain region of Japan was observed in recent work, which might provide information for evolutionary studies (Wang et al. 2015). The compatibility between different forms indicates that the elite genes in the wild could be easily used in modern breeding programs, while the genetic variations/differentiation among them will be helpful for their genetic and evolution study.

Considerable research has been done on the genetic diversity among cultivated adzuki beans. Although a high level of variations occurs in agronomic traits (Bai et al. 2014; Liu et al. 2009; Redden et al. 2009; Xu et al. 2008a), a fairly low diversity based on DNA molecular markers was observed (Wang et al. 2009a, b; Xu et al. 2008b; Yee et al. 1999). This is why most of the combinations used for map construction were derived from cultivated adzuki bean and its wild relatives.

1.3 Pests and Disease

Adzuki bean can be affected by a wide range of insects, including nematode pests. It has been reported that a total of 16 common insects attack adzuki bean in Japan.

The weevil, *Callosobruchus chinensis*, also called a bruchid, is the most serious and destructive pest of adzuki bean and its related crops in East Asia (Fernandez and Talekar 1990). Bruchid damage occurs from the field to the storage room by the

insect laying eggs on the seeds which then develop in the seeds. The weevil has a very short life cycle, usually four or more generations within one year (Liu et al. 1998), and one female weevil can lay hundreds of eggs in her lifetime, leading to rapid spread and heavy damage. People use chemicals for fumigation or physical methods, such as sun basking, to prevent damage. Although biological control is very safe and fast, the efficacy needs to be improved. Flower thrips and aphids are two other serious pests which can spread quickly. Flower thrips damages flowers preventing pollination or pod development. Aphids not only attack the young tender tissue, including stems, leaves and flowers or pods, but is the vector for diverse viruses. They can reach a high population in areas of suitable climate. Nematode attacks, leading to dwarf plants and fewer pods, has been observed in northern China, but are not usually serious in terms of production.

Earlier reporting suggests that two major diseases afflict adzuki bean in Japan: brown stem rot (*Phialophora gregata* Gams) and mosaic virus (Kim et al. 2014; Takahashi et al. 1998). Later studies found that brown stem rot is caused by the fungus *Phytophthora vignae* f. sp. *adzikucola*, first observed in Hokkaido, Japan 50 years ago and subsequently in Korea (Han et al. 1982; Kitazawa et al. 1978). High humidity will hasten the occurrence of stem rot. Mosaic virus also brings a certain yield loss, leading to dwarf plant and leaf atrophy. It can be prevented by using certain chemicals at the early stage, but finding resistant varieties is the best economic and most efficient solution for mosaic virus and other diseases and pests.

The common diseases on adzuki bean in China are powdery mildew, stem rot, mosaic virus and bacterial blight, but if the climate remains normal, these diseases are not serious and do not result in heavy loss of production with the use of chemical controls. However, if drought, waterlogging or high temperature occurs, both diseases and pests become worse.

1.4 Cultivation Limitations and Breeding Objectives

Adzuki bean is the second most important legume crop in Japan and both cultivation technology and breeding are much advanced. The cultivation of this crop is fully mechanized owing to the great successes in breeding (Jin 1994). All the breeding cultivars are erect with a high bottom pod, and lodging-resistant and suitable for mechanized harvest. However, as the main production area in Japan is in Hokkaido, the northern part of the country, where cold snaps occur irregularly, together with continuous cropping and frequent natural disasters, this leads to serious outbreaks of diseases, such as stem or root rot that can infect the soil. The present breeding objectives mainly focus on resistance to cold and diseases. In addition, since the usage of adzuki bean in Japan is as sweetened bean paste, a thin seed coat suitable for processing and appropriate starch granule size for good taste is desirable.

Most adzuki beans are cultivated under semi-mechanization in northern China, and segment harvesting is done by cutting machines and threshers modified from other crops are popular. In the other production areas, no mechanized operations are

Fig. 1.4 Growth variation exhibiting abnormal development of flower buds of adzuki bean, Baoding, China, 2012. (Photo courtesy of Lixia Wang 2011)

applied, leading to a higher cultivation cost and lower economic return. This situation mainly exists with small-scale cultivation systems which preclude mechanized operations. Therefore, the breeding objectives for the past decades have been for high yield, erect plants and early maturing, for labor saving and income improvement (Cheng and Wang 2009; Tian et al. 2004).

Changes in the agriculture structure in China, such as rural land transfer and in the crops cultivated, especially minor crops, have reached a substantial scale, such that the government has offered special financial support to improve the levels of mechanization. Therefore, new varieties of adzuki bean with characters suitable for mechanized operations are in urgent need. In addition, with the climatic fluctuations in recent decades attributed to climate change, such as drought, flood and high temperature, cultivars with resistance to natural disasters should also be developed. For example, we observed an abnormal development of flower buds at Baoding, China in 2012, where most of the adzuki bean flower buds developed into leaflets, leading to a complete loss for the season (Fig. 1.4). Another abnormal phenomenon was found at Shijiazhuang, China in 2018, where the entire plants of some varieties were yellow in color from the seedling stage, leading to a heavy decrease in yield (Fig. 1.5). The exact cause of these abnormalities is not known but is suspected to be related to climate change.

In Korea, there is almost no mechanization of adzuki bean cultivation and farmers sometimes harvest adzuki bean by segment harvesting using cutting machines and threshers. The breeding programs focus almost exclusively on the early maturity, disease resistance, high yield and good quality.

1.5 Conventional Breeding

Most historic and current varieties of adzuki bean were developed by pedigree selection and hybridization. Pedigree selection was performed from a very early stage in Japan. A series of famous large-seeded and early-maturing cultivars were

Fig. 1.5 Abnormal yellowing appearance of the entire plant of adzuki bean, Shijiazhuang, China, 2018. (Photo courtesy of Lixia Wang 2018)

developed, such as *Toyomi dainagonn* and *Akane dainagonn*. Hybridization was then used as the main method in adzuki breeding program for a long period, especially at the Hokkaido Prefectural Agricultural Experiment Station, which was designated as the national breeding center of Japan in the middle of the last century. *Erimo syouzu*, with high yield, cold tolerance and good bean quality, is the most widely planted cultivar in Japan. For resistance breeding, scientists also obtained great results. For example, cultivars resistant to brown stem rot were released at the end of last century (Fujita 2007) and *Yeonkeum*, a cultivar with green seed, is reported to be resistant to mosaic virus (Moon et al. 2006).

From the *Records of Chinese Food Legumes Cultivars* (Cheng and Wang 2009), we know that the formal breeding on adzuki bean began from the end of the 1970s in China, following the nationwide collection of genetic resources. It took a long period of time for the breeding methods to change from pedigree selection to hybridization. Early registered cultivars, such as *Zaohong 1*, *Zhonghong 3* and *Jingnong 2* were all selected from local landraces, while *Pinhong Youzi 961* and *Pinhong Youzi 962* were selected from the introduced adzuki bean from Japan at the end of the twentieth century. The most efficiently used parents for hybridization in adzuki bean are cvs. *Akane dainagonn* and *Taiseikou*; cvs. *Jihongxiaodou 4* and *Jihong 8937* are examples of such hybrids. As adzuki bean is light and temperature sensitive, many locally-adapted cultivars, such as *Baihong 1*, *Jingnong 5, Pinhong Youzi 611* and *Jinxiaodou 1*, showing a good erect growth habitat, were developed and released (Fig. 1.6). In total, about 100 varieties have been released in China so far, and one-half of those were hybrids using genetic material from Japanese cultivars (Cheng and Wang 2009; Wang et al. 2009a, b).

Fig. 1.6 The appearance of different local varieties. (Qinsheng Cai 2012)

Adzuki bean breeding in Korea has also recorded achievements; for example, *Seagil*, a landrace, is specially used to make New Year cakes. *Chilbo* cv., developed using hybridization, is black-seeded and high-yielding, while *Kyungwon* cv. is early-maturing and lodging-resistant (http://genebank.rda.go.kr/eng/EgovPageLink.do). The major cultivars in China, Japan and Korea, with their important traits, are listed in Appendix II.

1.6 Biotechnology

1.6.1 Genetic Map and Gene Cloning

Similar to other *Vigna* crop plants, genetic studies of adzuki bean are lagging behind, including the construction of genetic maps and gene mapping. The reasons for this may be: first, low genetic variation makes it difficult to obtain more polymorphic markers (Kaga et al. 1996, 2000) or second, adzuki bean is a minor crop and is not widely cultivated, compared with other major crops, and hence receives less funding for breeding research.

Most of the reported genetic maps were constructed using combinations between cultivated adzuki bean and its wild relatives (Table 1.2). The first map of adzuki bean was constructed using an F_2 population derived from adzuki bean and *Vigna nakashimae* with only 132 markers mapped (Kaga et al. 1996). The map (Fig. 1.7) was constructed based on a cross between adzuki bean and rice bean with 188 markers (Kaga et al. 2000). Both maps consisted of 14 linkage groups. With the development of microsatellite markers, the number of linkage groups reached 11, in agreement with the number of chromosomes, and the density of the genetic map is much higher with an average distance between markers of 1.85 cM (Han et al. 2005). The latter two sets of maps were also constructed mainly using SSR markers with a fairly low density (Isemura et al. 2007; Kaga et al. 2008). From the density of maps, slow progress on the genetic study of adzuki bean can be seen. An SNP map, consisting of 2032 markers, was constructed using a RIL population derived from adzuki bean and *V. nipponensis*. The density of this map is much higher than those in earlier reports, but the application and detection of SNPs were quite complicated and costly. Thus, more polymorphic PCR-based markers are needed that could be simply tested using gel analysis.

Bruchid resistant genes were analyzed in adzuki bean using a population derived from adzuki bean and *Vigna nepalensis* and relative QTLs were identified (Somta et al. 2008), but no further study has yet been published. Using a similar combination, traits related to domestication were mapped as well, and the results showed that these traits always clustered together on the chromosomes (Isemura et al. 2007). Although both the map construction and gene mapping are lagging, there are reports of gene isolation of adzuki bean. For example, genes controlling the synthesis of starch (Peterbauer et al. 1999), coding abscisic acid-specific glucosyltransferase (Xu et al. 2002) and resistance to bruchids (Chen et al. 2005) or mosaic virus (Chen et al. 2009), have been cloned and analyzed.

Table 1.2 Description of the published genetic maps of adzuki bean

Combination	Population type	Population size	Marker type	Number of markers	Number of linkage groups	Total length	References
Vigna angularis× *V. nakashimae*	F2	80	RAPD, RFLP, morphological marker	132	14	1250 cM	Kaga et al. (1996)
V. angularis× *V. umbellata*	F2	86	RAPD, RFLP, morphological marker	189	14	1702 cM	Kaga et al. (2000)
V. nepalensis× *V. angularis*	BC1F1	187	SSR, AFLP, RFLP	486	11	832.1 cM	Han et al. (2005)
V. nipponensis× *V. angularis*	F2	188	SSR, STS, CAPS, SCAR, AFLP, morphological marker	233	10	771.9 cM	Kaga et al. (2008)
V. nepalensis× *V. angularis*	F2	141	SSR	74	11	649.7 cM	Isemura et al. (2007)
V. nipponensis× *V. angularis*	RIL8	153	SLAF	2032	11	1628.15 cM	Liu et al. (2016)

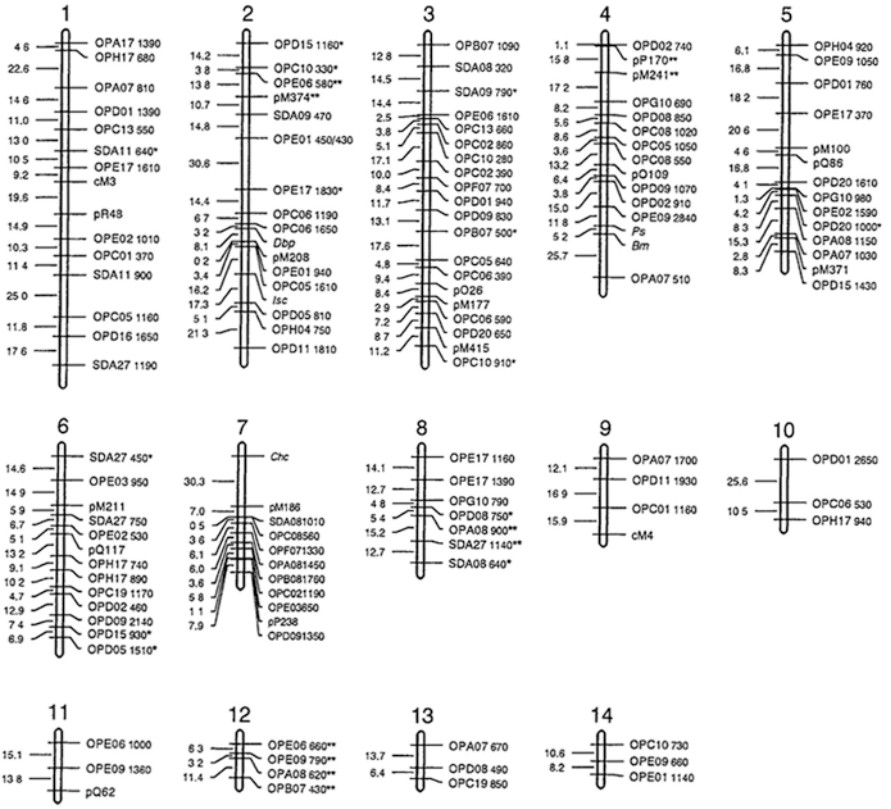

Fig. 1.7 The first linkage map of adzuki bean constructed with a F_2 population derived from *Vigna angularis* x *V. nakashimae*. (Source: Kaga et al. 1996)

1.6.2 Tissue Culture and Genetic Engineering

Genetic and genomic studies are basic knowledge for the application of biotechnology in breeding, including marker-assisted selection (MAS) and genetic modification. MAS is seldom reported in breeding programs of adzuki bean, although it might be used in some breeding facilities. However, no cultivar has yet been reported and released using transgenic methods.

Tissue culture, a key step to obtain transgenic plants, has been reported (Gao et al. 2013; Lu et al. 1985; Sato et al. 2008). An early study suggested that trans-Zeatin (ZT) was better than 6-Benzylpurine (6-BA) for adventitious bud growth from callus of the embryonic axis (Xu et al. 1984), while a high concentration of 6-BA could boost bud differentiation from callus (Jin and Futsuhara 1993) and a combination of hormones was helpful for elongation of adventitious buds of adzuki bean hypocotyls (Chen et al. 2011). Based on earlier tissue-culture studies, the transformation of different genes into adzuki bean was performed (Fig. 1.8). This

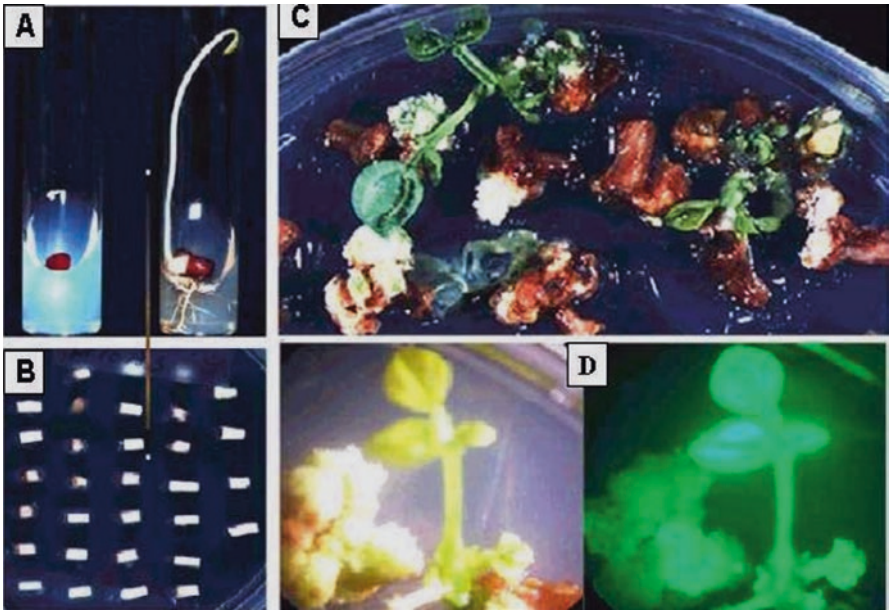

Fig. 1.8 Genetic transformation of azuki bean. (**a**) Seed germination (left) and elongated epicotyle in dark (right), (**b**) Cocultivation of epicotyl explants with *Agrobacterium* strain EHA105, (**c**) Shoot regeneration from organic calli induced on the selection medium, (**d**) Transgenic shoot screened for GFP expression under white light (left) and blue light (right). Source: Khalafalla et al. (2005)

includes the neomycin phosphotransferase II gene (npt II) together with reporting gene (Khalafalla et al. 2005; Yamada et al. 2001) and disease resistant gene *VaPR3* (Chen et al. 2011). In spite of the final transformation ratio, low or high, these results really establish an important foundation in the expression regulation mechanisms and the development of genetic improvement. However, further research should be enhanced to accelerate the molecular breeding of adzuki bean.

1.7 Conclusions and Prospects

Adzuki bean is a healthy and nutritional food traditionally consumed in Asian countries. Both the genetic resource collections and breeding of this species have made great achievements in recent years. This has permitted the release of a series of elite cultivars for production which has boosted the cultivation level and economic benefits. Owing to the frequent occurrence of natural disasters and climate change, leading to plant diseases and pests or adversity becoming worse, more resistant/tolerant cultivars are urgently needed. For example, cultivars resistant to continuous cropping and tolerant of cold are most important in Japan, especially in its northern

region, which is the main production area of adzuki bean. Drought-tolerant cultivars in northern and water-tolerant cultivars in southern China are particularly required, given the occurrence of unusual weather patterns in recent decades. Although resistance breeding has been improved in the past, to strengthen the horizontal resistance of cultivars, more resistant gene resources are needed. Aside from resistance breeding, quality breeding has good prospects as well, with the nutritional factors revealed to be contained in the adzuki bean (Kim et al. 2016).

Wild adzuki beans and/or the wild relatives of cultivated species that have undergone a long history of natural disasters and have persisted in the face of diseases and pests, may contain many potential elite genes that could represent rich resources for adzuki breeding (Iseki et al. 2016, 2018). The traditional way to use the genes of wild species is hybridization and reproductive isolation can even be solved, using embryonic rescue. However, conventional methods of breeding have low efficiency and are time-consuming and cannot meet the changes of diverse demands from production and markets. Modern gene editing biotechnology could greatly accelerate the breeding process by employing molecular operations. However, thus far these have been little used in adzuki bean breeding, mainly because research on this crop has lagged behind. With the release of the whole genome sequence of adzuki bean, we believe that the genetic and genomic studies on adzuki bean will experience rapid progress in the coming years.

Another way to obtain resistant genes is through mutation, which has been successfully used in crop breeding. The use of conventional mutation has limited efficacy in the identification of desirable traits, because mutations by chemical or physical method are usually random. A new technology, directed mutagenesis, can lead to much more efficient mutations, based on the development of genetic manipulation at the DNA molecular level. Therefore, we hope that genetic and genomic studies on adzuki bean will achieve rapid progress in the near future, to support the use of gene mining/editing in breeding.

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Appendices

Appendix I: Research Institutes Concerned with Adzuki Bean

Institute	Specialization and research activities	Contact information and website
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences	Germplasm collection, breeding and molecular markers	Dr. Lixia Wang Wanglixia03@caas.cn http://ics.caas.cn/

(continued)

Institute	Specialization and research activities	Contact information and website
Kasetsart University	Genetic study and gene mapping	Dr. Prakit Somta agrpks@ku.ac.th http://www.ku.ac.th
Genetic Resources Center, National Agriculture and Food Research Organization	Wild germplasm collection and classification	Dr. Norihiko Tomooka tomooka@affrc.go.jp http://www.naro.affrc.go.jp
Hokkaido National Agricultural Experiment Station	Breeding	Dr. Sato Hitoshi satohhs@agri.pref.hokkaido.jp https://www.hro.or.jp/list/agricultural/research/tokachi/
Hokkaido University	Resistant study and breeding	Dr. Jun Abe jabe@res.agr.hokudai.ac.jp https://www.global.hokudai.ac.jp
Plant Genomics and Breeding Institute, Seoul National University	Genomic study	Dr. Suk-Ha Lee sukhalee@snu.ac.kr https://www.useoul.edu/
The World Vegetable Center	Germplasm collection and breeding	Dr. Ram Nair ramakrishnan.nair@worldveg.org https://avrdc.org/

Appendix II: Genetic Resources of Adzuki Bean

Cultivars	Important traits	Cultivation location
Zhonghong 4	Good quality, resistant to mosaic virus and drought	China
Zhonghong 5	Good quality, suitable for bean paste	China
Jingnong 5	High yield, resistant to rust and powdery mildew	China
Jihong 352	Early mature, resistant to mosaic virus and leaf spot	China
Jihong 9218	Early mature, high yield, resistant to mosaic virus and leaf spot	China
Baihong 6	High yield, resistant to mosaic virus and root rot	North China
Jihong 6	High yield, resistant to leaf spot, drought and lodging	North China
Baohong 947	Good quality, high yield, wide adaptability	North China
Erimo syouzu	Good quality, high yield, cold tolerant, suitable for processing	Japan
Kitaromann	Early mature, resistant to stem rot, blight, lodging	Japan
Kitano otome	Resistant to stem rot/blight/lodging	Japan
Kotobuki syouzu	Resistant to stem rot	Japan

(continued)

Cultivars	Important traits	Cultivation location
Syumari	Resistant to stem rot/blight, good quality	Japan
Kitaasuka	High yield, resistant to stem rot	Japan
Kitahotaru	White seed, resistant to stem rot/blight, suitable for processing	Japan
Toyomi dainagonn	Large seed, high yield, cold tolerant	Japan
Chungju	Resistant to mosaic virus and powdery mildew	Korea
Jungbu	High yield	Korea
Chilbo	Black seed, high yield	Korea
Kyungwon	Early mature, resistant to lodging	Korea
Seagil	Consistent maturity, special use for New Year cakes	Korea

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Chapter 2
Recent Advances in Breeding, Marker Assisted Selection and Genomics of Black Gram (*Vigna mungo* (L.) Hepper)

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Abstract Black gram (*Vigna mungo* (L.) Hepper) is an important leguminous pulse crop, which is grown for its protein-rich edible seeds. Due to a short life cycle and N-fixing ability, this crop is also grown as an intercrop and catch crop. Generally, exotic lines and cultivated germplasm have been used for genetic improvement of *V. mungo*. However, lack of suitable ideotypes for variable cropping systems, low harvest index, abiotic/biotic stresses and unavailability of quality seeds of improved varieties remain major constraints to achieve the true yield potential of this crop. This chapter presents a comprehensive worldwide overview of available biodiversity in *V. mungo*. Moreover, a detailed record is also presented for mutation breeding and recent advances in molecular marker-assisted breeding and genomic research for black gram with emphasis on genetic linkage maps, genes/QTLs mapping, genetic engineering and hybridization for improvement of agronomically-important traits. Availability of genomic resources which can be used to accelerate molecular breeding in *V. mungo* is also discussed.

Keywords Black gram · Molecular breeding · Mutation breeding · Next-generation sequencing

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2.1 Introduction

Among the legumes, the genus *Vigna* includes more than 200 species, which are dispersed in different regions of the Americas, Australia, Africa and Asia. Among them, 9 species have been domesticated as important food crops. These consist of *V. vexillata* (L.) (zombi pea); *V. reflexo-pilosa* Hayata (créole bean); *V. subterranea* (L.) Verdc. (Bambara groundnut); *V. angularis* (Ohwi) Ohwi & Ohashi (azuki bean); *V. unguiculata* (L.) Walp. (cowpea); *V. radiata* (L.) Wilczek (mung bean); *V. umbellata* (Thunb.) Ohwi & Ohashi (rice bean); *V. aconitifolia* Jacq. (moth bean) and *V. mungo* (L.) Hepper (black gram) (Chaitieng et al. 2006). The tubers, pods and seeds of these plants are excellent human sources of carbohydrates, amino acids, minerals and vitamins. Moreover, numerous wild *Vigna* species are cultivated as supplementary food or ground cover (Chaitieng et al. 2006; Maréchal 1978). In industrial and economic terms, the three most important *Vigna* species are mung bean (*V. radiata*), cowpea (*V. unguiculata*) and black gram (*V. mungo*). Cowpea is largely cultivated in Africa, whereas mung bean and black gram are mostly grown in Asia.

Black gram (also called mash or urd bean) is thought to have been domesticated in the Indian subcontinent from a wild ancestor, *Vigna mungo* var. *silvestris* Lukoki, Marechal & Otoul (Chandel et al. 1984). According to the archeological evidence, *V. mungo* was domesticated in about 2500 BC (Fuller and Harvey 2006). Plants of *V. mungo* are erect, fast-growing annual and herbaceous legumes reaching a height of approximately 30–100 cm. They have well developed taproots, diffusely branched stems and trifoliate leaves. The inflorescence is formed at the tip of peduncle and produces small, yellow-colored papilionaceous flowers. Plants bear hairy pods with short hooked beaks. A pod usually carries 4–10 ellipsoid seeds that are mottled to black in color (Jansen 2006; Sen and Jana 1964). Black gram is a self-fertilized crop, and has a 3-month life cycle (Jansen 2006). Seeds are an important source of dietary proteins, fiber, amino acids, low saturated fatty acids and possess high antioxidant capacity (Zia ul Haq et al. 2014), and are consumed in soups and made into flour used in the food industry. Moreover, black gram sprouts provide an important vegetable source of minerals and vitamins. In Japan and Thailand, black gram sprouts are preferred over the mung bean sprouts for their longer shelf life. This plant is primarily cultivated in Asian countries such as India, Afghanistan, Bangladesh, Nepal, Philippines, Pakistan, Thailand, Sri Lanka and Myanmar. Black gram has characteristic features like a short life cycle, drought tolerance and atmospheric nitrogen-fixing ability (in association with *Bradyrhizobium* and *Rhizobium* bacteria), which make it an important component of several cropping systems such as wheat and rice.

Although there are no official data on cultivated area of *Vigna mungo*, the total area is estimated to be more than five million ha. India is the chief producer (around three million ha), followed by Myanmar (approximately one million ha) and then Pakistan (some 0.5 million ha). The major limitations for genetic improvement of black gram include lack of suitable ideotypes for variable cropping systems, low harvest index, exposure to abiotic and biotic stresses and unavailability of quality

seeds of improved varieties and lack of exploitable genetic variability. This is primarily due to the repeated use of only a few parents with a great degree of relatedness in crossing programs (Jayamani and Sathya 2013).

This chapter describes current information regarding available genetic diversity, breeding approaches, molecular breeding and genomics that are currently in practice to improve *Vigna mungo* crop performance within a genetic perspective.

2.2 Genetic Diversity

In *Vigna mungo*, germplasm biodiversity originated and flourished because of different factors such as genetic drift, migration and mutation following adaption and speciation (Maestri et al. 2002). The loss of germplasm diversity is the foremost risk for the conservation and adaptive potential of any species. Germplasm diversity is considered vital for plant breeding to understand quantitative traits and phylogenetic relationships (Sohel et al. 2016). The characterization of the gene pool is an important part of germplasm management and future use (Ganguly and Bhat 2012). Moreover, proper utilization of genetic diversity is considered crucial for a steady increase in yield, even under disease epidemics and unfavorable environmental conditions.

To enhance the availability of *Vigna mungo* as a source of feed and food, the germplasm diversity of this crop must be analyzed. As compared to mung bean or cowpea, black gram as a crop is given relatively less attention for analyzing germplasm diversity. However, there are some reports of genetic diversity of black gram for agronomic and morphological traits (Ghafoor et al. 2001; Shafique et al. 2011; Choudhary et al. 2018), branches or pods per plant (Patidar et al. 2018), seed stored protein (Ghafoor and Ahmad 2005) and resistance against biotic stress (Vishalakshi et al. 2017). Most of these studies have used SDS PAGE (Tripathy et al. 2016), isozyme markers (Singh and Singh 2006), AFLP (amplified fragment length polymorphism), RAPD (random amplified polymorphic DNA), ISSR (inter simple sequence repeat) and SSR (simple sequence repeat) (Ghafoor et al. 2012; Gupta and Gopalakrishna 2009; Kaewwongwal et al. 2015; Souframanien and Gopalakrishna 2004; Sivaprakash et al. 2004) and ISSR and SCAR (sequence characterized amplified region) (Souframanien and Gopalakrishna 2006) to analyze germplasm diversity and to identify desired genotypes (Table 2.1; Fig. 2.1). However, these studies have provided limited information of black gram genetic diversity because many of the studies used a small number of genotypes, mostly belonging to a particular geographic area. Furthermore, RAPD, AFLP and ISSR markers are generally dominant in nature, making them less appropriate for diversity analysis (Lynch and Milligan 1994). Recently, Kaewwongwal et al. (2015) studied 534 (14 wild and 520 domestic) accessions from several geographic areas related to major cultivation regions using SSR markers. They proposed that *V. mungo* was naturalized to Africa and America from different areas of the Indian subcontinent which is the epicenter of domestication.

Table 2.1 Summary of germplasm evaluation studies involving morphological and molecular markers in *Vigna mungo*

Trait	Number of genotypes	Method of analysis	References
12 agronomic characters	29	ANOVA	Gupta et al. (2008) and Patidar et al. (2018)
Drought tolerance	69	ANOVA	Kundu et al. (2017) and Iseki et al. (2018)
	7	ANOVA	Iseki et al. (2018)
Resistance against urd bean leaf crinkle disease	87	Visual observation of infection	Binyamin et al. (2011) and Gautam et al. (2016)
Salt tolerance	69	Cluster analysis	Iseki et al. (2016) and Win and Co (2017)
Genetic diversity	20	AFLP	Gupta and Gopalakrishna (2009)
Selection against multiple disease resistance	12	RAPD	Vishalakshi et al. (2017)
Genetic diversity	3	RAPD	Win et al. (2016)
Interspecific recombinants	36	SSR, RAPD	Abbas et al. (2015)
Genetic diversity	534	SSR	Kaewwongwal et al. (2015), Tripathy et al. (2016), and Jeevitha et al. (2018)
Resistance to yellow mosaic virus	12	SSR	Sehrawat et al. (2016) and Ganguli et al. (2016)
Genetic diversity	1	Genic-SSR markers	Souframanien and Reddy (2015) and Suman et al. (2018)
Immature seed transcriptome	10	Genic-SSR	Souframanien and Reddy (2015)
Genetic variations	3	ISSR	Wahlang et al. (2019a, b)
Mung bean yellow mosaic virus (MYMV) resistance	14	ISSR, SCAR	(Souframanien and Gopalakrishna (2006)
Phylogenetic assessment	24	Morphological, nrDNA ITS2	Bhavisha et al. (2019)
Genetic diversity	466	SNP	Noble et al. (2018)
Resistance to bruchid beetle (*Callosobruchus maculatus*)	11	SNP-based approach	Somta et al. (2019)

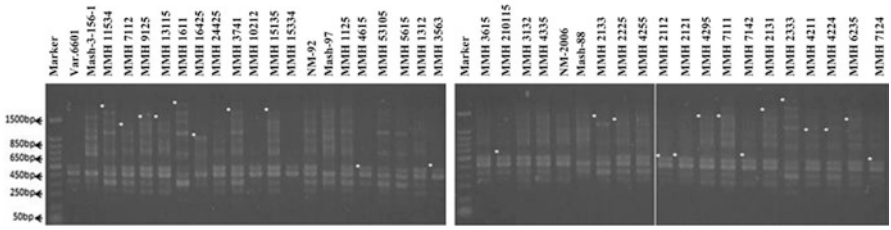

Fig. 2.1 PCR profiles of parental genotypes along with interspecific recombinants using RAPD primer OPS-07. *Marker for recombination. (Source: Abbas et al. 2015)

2.3 Hybridization

In traditional *Vigna mungo* growing areas there is a dire need to enhance its agronomic yields to fulfill the demand of an ever-growing population. However, the recalcitrant nature of *V. mungo* seed and the lack of highly efficient regeneration methods, make it difficult to improve this crop through direct genetic transformation. Fortunately, conventional breeding offers diverse choices for reducing limitations for improvement of this crop. Additionally, the existence of narrow genetic variability, deficiency of appropriate ideotypes for diverse cultivation systems and a low harvest index are some other factors which may limit *V. mungo* crop improvement. Therefore, introgression or interspecific hybridization becomes an important approach to introduce desired traits or genetic variability into this crop.

According to Bhanu et al. (2018), use of related species of *Vigna mungo* should be a promising approach to increase the genetic diversity and to expand the genetic background of cultivated germplasm of this species. The rice bean (*V. umbellata*) appears as a competent donor parent to incorporate resistance against MYMV and numerous yield-related traits in *V. mungo* (Sehrawat et al. 2016). Introgressive hybridization of alien gene(s) from wild parents or relatives would not only lower the negative effects of biotic or abiotic stresses, but would also enhance the yield and quality of the crop. Consequently, such pre-breeding practices are required, which specifically involve wild/cultivated *Vigna* species carrying beneficial genes related to quality, yield and stress resistance. Moreover, it is highly desirable to collect specific information about the crossability of diverse parents for the isolation of rare genetic recombinations related to improved disease resistance and enhanced crop yield (Bhanu et al. 2017a). Several factors contribute to successful breeding programs, including selection of superior parents for hybridization and highly specific knowledge of the trait inheritance system or mechanism. It has always been possible to improve a particular crop by simply integrating wild genes into the cultivated varieties.

Available gene pools of *Vigna* species could be used systematically to develop specific hybrids through interspecific crosses (Abbas et al. 2019; Dikshit et al. 2007). Such introgressed hybrids may serve as an additional gene pool resource. However, the incompatibility of these species significantly limits the possibilities of desirable gene transfer. Black gram possesses several important features like synchronous maturity, dwarf plant size, better branching, non-shattering pods and biotic stress resistance (Abbas et al. 2015). Similarly, mung bean has several desirable traits such as large and higher number of seeds per pod, erect growth habit, early maturity and other quality traits (Tickoo et al. 2006). Rice bean on the other hand has numerous characters such as number of clusters per plant, large number of bold seeds per pod, long pods and resistance against *Cercospora* leaf spot (CLS) or mung bean yellow mosaic virus (MYMV). Black gram, rice bean and mung bean plants flower in stages with terminal or axillary racemes having clusters of 10–20 cleistogamous flowers. Shedding of flowers is common and may reach an average of almost 60%. Depending on the cultivar and season, 2–5% outcrossing may take

place. Based on early studies, Bhanu et al. (2018) proposed a hybridization method to successfully achieve the interspecific hybridization in different species of *Vigna*.

2.3.1 Crossing Ability Studies

Interspecific hybrids have been attempted by several researchers (Bhanu et al. 2017a,b; Chowdhury and Chowdhury 1977; Pandiyan et al. 2010; Shanmungam et al. 1983; Singh and Singh 1991, 2006; Singh et al. 1997; Subramanian 1980). However, the F_1s were found to be sterile or partially fertile. The reasons behind this cross failure in legumes are not understood yet. In some of these reports, pollen tubes were not able to penetrate the style and stigma, while in other cases, fertilization happened but embryo abortion occurred during the process of embryogenesis. Among all, embryo degeneration was observed as the principal cause of interspecific hybridization failure in food legumes. Meanwhile, a variable rate of success is observed in hybridization, which fluctuates from 2.3–43.24% (Bhanu et al. 2017b; Singh et al. 1996). In addition to the abovementioned pre- and post-fertilization restrictions, there are other reasons as well for these poor crosses. These include the polymorphism of genes involved in crossability (Snape et al. 1979), stigmatic exudates of the *Vigna mungo* (Shanmungam et al. 1983), and, most importantly, weather conditions (Bhanu et al. 2017b). There are several factors that affect the efficiency of crosses. To develop successful hybrids, one of the most important prerequisites is the good percentage of pod setting after crossing. Pod setting and flowering are affected by genetic response, photoperiods, wind speed, temperature, rainfall and relative humidity (Talukdar and Shivakumar 2012). Moreover, during artificial pollination the rate of flower drop is also increased, making crossing even more difficult. *Vigna mungo* and *V. radiata* possess identical timing for anthesis (0500–0800), anthers dehiscence (10–14 h before anthesis) and stigma receptivity (from anthesis time to 6–8 h post anthesis). However, the average style length is variable.

2.3.2 Polyploidy

The term *ploidy* refers to the sets of homologous chromosomes in a living cell. Accordingly, monoploid (with single or *n* set), diploid (with double or *2n* sets) and polyploid (with more than two sets) organisms exist in nature (Crespel and Meynet 2003). There are two categories of polyploidy: autopolyploidy (when a single parental genome is doubled) and allopolyploidy (when a hybridized genome is doubled) (Aversano et al. 2012). Autopolyploidy has been identified as a relatively abundant mechanism in nature by which additional numbers of genome copies are developed resulting in evolutionary and genetic novelty to the organism. As such, it is considered to be a pathway for adaptation and has played an important role in

plant speciation. Autopolyploids are characterized by improved genome flexibility, permitting them to adapt and persevere across heterogeneous lands over the long term.

Blakeslee and Avery (1937) used colchicine for whole genome doubling in plants. Later, it was used to induce polyploidy in diploid and interspecific hybrids to produce synthetic auto- and allopolyploids (Chen and Ni 2006). Colchicine promotes the disruption of spindle formation, which thwarts chromosomal disjunction and cell division (Ye et al. 2010). For induction of colchitetraploids, the apical growing point of cotyledons from germinated seedlings are treated with 0.15–0.2% w/v aqueous colchicine solution (Wahlang et al. 2019a). In view of the significance of autopolyploidy, Rao and Raina (2005) performed a detailed cytological evaluation of colchitetraploids (tetraploids produced after the duplication followed by colchicine application) in C_1 generations of *Vigna radiata, V. unguiculata* and *V. mungo*. Recently, Wahlang et al. (2019a) performed cytological evaluation for three successive generations (C1, C2, C3) in *V. radiata* and *V. mungo*. The diploid number of chromosomes in diploids of both species were 2n = 2× = 22. This study found that the chromosome number of colchitetraploids was 2n = 4× = 44 and it was stable in successive generations. Wahlang et al. (2019b) also investigated the genetic variations associated with artificial somatic autopolyploids in *V. mungo*. The population of autopolyploids demonstrated some degree of genetic heterozygosity in comparison to their diploid counterparts. Further, polymorphic ISSR-DNA loci were identified among the colchitetraploids. These regions were used to design SCAR markers for the identification of colchitetraploids versus the putative diploids of *V. mungo (*Wahlang et al. 2019b). Since polyploidy is associated with a number of commercial benefits including larger fruits and seeds, seedlessness, better disease resistance and the fact that most of major agriculture crops (wheat, maize, oats, alfalfa, sugarcane, potato, sweet potato, banana, cotton) are polyploids (Dar and Rehman 2017). Polyploidy has a bright future in improving *V. mungo* performance in terms of agronomic yield, stress tolerance and quality enhancement.

2.4 Mutation Breeding

Induced mutations play a remarkable role in plant breeding and genetics by contributing an enormous amount of genetic variability. The global applications of mutation techniques in plant breeding programs have helped to develop thousands of important varieties in numerous crop species, and generating billions of dollars of additional revenue (Raina et al. 2016). An affiliation of genetic diversity between genotypes and major yield traits is of prime importance for numerous breeding programs. Various approaches have been adopted for induced mutagenesis in *Vigna mungo* (Table 2.2) involving chemical and physical methods. Studies on heritability, genetic variability and genetic advance of grain yield traits in *V. mungo* indicate the scope of improvement through selection (Baisakh et al. 2014). Induced mutations were also found to be effective in creating genetic variability for quantitative traits

Table 2.2 Different mutagens used and traits identified in *Vigna mungo* to enhance different traits

Serial #	Mutagen used	Traits improved	References
1	EMS (ethyl methane sulfonate)	Chlorophyll	Usharani and Kumar (2015), Patial et al. (2017), and Veni et al. (2017)
		Protein content, nitrate reductase activity	Thilagavathi and Mullainathan (2011)
		Methionine content	Arulbalachandran et al. (2009a, b)
		Number of seeds, number of primary branches and plant height	Usharani and Kumar (2016) and Kuralarasan et al. (2017)
		Seed yield	Verma et al. (2018)
2	Gamma rays	Chlorophyll	Usharani and Kumar (2015) and Ramchander et al. (2017)
		Methionine content	Arulbalachandran et al. (2009a, b)
		Pods per plant, number of seeds per plant, number of primary branches, pod length	Usharani and Kumar (2016) and Anittha and Mullainathan (2018)
		Seed yield	Raina et al. (2018)
3	Sodium azide	Leaf length, plant height	Baba (2015)
		Chlorophyll	Raina et al. (2018)
4	Diethyl sulfate	Chlorophyll, male sterility, long pod	Anittha and Mullainathan (2018)
5	Colchicine	Chlorophyll, male sterility, long pod	Arulbalachandran et al. (2009a, b)
6	Electron beam	Chlorophyll	Thilagavathi and Mullainathan (2011) and Veni et al. (2017)

like chlorophyll content, protein content, plant height, number of primary branches, pod number, pod length, number of seeds per pod and seed yield in *V. mungo* (Table 2.2). A double-sized seed mutant was developed by gamma rays treatment to cv. Phitsanulok 2 (known as BS 48) (Chinchest and Nakeeraks 1990). The mutant plant also has larger stems and leaves, therefore is was termed a *multiple organ gigantism* (*mog*) mutant. Later, a linkage map was developed employing BC_1F_1 population originated from *mog* and wild accession TC2210 (Chaitieng et al. 2006). Recently, Naito et al. (2017) employed a map-based cloning technique and effectively identified a deletion of eight base pairs in the coding region of the *VmPPD* gene. This gene is a homologue of the *PEAPOD* (*PPD*) gene in *Arabidopsis*, which is involved in a process of cell division arrest of meristematic tissue. However, other mutations were not detected in wild type sequencing or in adjacent gene sequences. Consequently, it is highly valuable to mutate *PPD* genes for developing *mog* phenotype for use in breeding programs to increase seed size. This may directly increase the commercial and economic value of grain legumes (Naito et al. 2017).

2.5 Molecular Breeding

Several conventional breeding methods such as the pedigree method, bulk or mass selection, backcrossing techniques or relevant modified methods have been adopted to develop new cultivars of black gram (Keneni et al. 2011). Such methods rely entirely on the number of genes for a particular trait and mode of inheritance. However, mutation breeding, pure line selection, hybridization/recombination breeding and direct introductions have been effectively implemented for producing new varieties of *Vigna mungo* and *V. radiata* for several traits (Fernandez and Shanmugasundaram 1988; Kaewwongwal et al. 2015; Singh et al. 2011; Tickoo et al. 2006). However, productivity in orphan legumes like *V. mungo* is low and has been stagnating for the last few decades. A large number of abiotic and biotic factors affect the quality and yield potential of these crops. Therefore, the prime breeding objective is to cope with these stresses and increase the productivity.

Until recently, a number of morphological and biochemical markers were used to trace the inheritance of a target gene (Table 2.1). Shafique et al. (2011) used morphological markers and SDS-PAGE (to determine variation in seed storage proteins) for evaluating the genetic diversity of 34 *Vigna mungo* cultivars. It was observed that dry pod weight, number of branches per plant and biological yield exhibit the highest level of coefficient of variation. Recently, Gurumurthy et al. (2019) investigated the response of drought stress on morpho-physiological and biochemical features of *V. mungo* genotypes. It was suggested that stomatal conductance, rate of transpiration, photosynthesis, peroxidase activity and proline content may be used to estimate drought tolerance in this crop. However, these markers are restricted in number and are strongly influenced by ecological factors. One of the most significant developments in biology is the detection and analysis of naturally occurring DNA sequence variation using DNA markers or molecular markers, which have numerous advantages over biochemical and morphological markers. These molecular markers provide an indispensable tool to develop genetic linkage maps and to tag agronomically-important traits for use in marker-assisted selection (MAS), collectively known as *molecular breeding* (He et al. 2014). This term is also used to include several novel strategies comprising genomic selection (GS), marker assisted recurrent selection (MARS), marker-assisted backcrossing (MABC) and genome-wide selection (GWS) (Ribaut et al. 2010). MAS is regarded as a powerful methodology and novel strategy for genetic improvement of plants, and up to now widely used in multiple crop species (Jiang 2013; Xu 2010). During the last decades, DNA based markers system like RAPD, AFLP, RFLP, SSR, DArT and SNPs have become available (Jiang 2013; Kumar et al. 2016). Of these markers, AFLP, RAPD and RFLP are mostly employed for diversity analysis and marker-assisted breeding in pulses, but conventional plant breeders discourage their use in MAS because of their difficulty in handling, poor reproducibility and the requirement for skilled person for identifying and gathering markers (Gupta and Gopalakrishna 2009). Plant breeders prefer PCR-based markers (SSRs, SNPs), because they are more effective

for genotyping segregating plant populations with minimum infrastructure facilities and in a cost-effective manner (Saxena et al. 2010; Varshney et al. 2009).

Moreover, coding genome sequences, functionally characterized genes and ESTs (expressed sequence tag) have been used to design molecular markers, conserved orthologous sets, SNPs and SSRs (Varshney et al. 2005). These markers are also known as functional markers developed from putative exons and have complete linkage with the gene (Young and Udvardi 2009). Currently, functional genomic resources (genes or ESTs) are accessible for several pulses like cowpea, pigeon pea, chickpea and soybean (Choi et al. 2004; Kumar et al. 2016), which can facilitate the development of functional markers in black gram (*Vigna mungo*). For example, the sequence of candidate resistance gene and resistant gene analogues (RGA) have been converted into molecular markers as well as RGA polymorphism. However, the deficiency of molecular markers and genomic information restrict genetic improvement of *V. mungo.*

In an attempt to identify the QTL for MYMV resistance in *Vigna mungo*, a RIL population was used for molecular tagging of the MYMV-resistance gene. The RIL population was developed from the crossing of *V. mungo* (cv. TU94-2) and *V. mungo* var. *silvestris*. In this study, an ISSR marker (ISSR8111357) was mapped at 6.8 cM away from the MYMV-resistance gene. Besides, the effectiveness of this marker was evaluated in MYMV-susceptible and resistant lines. Later, the same marker was developed to a SCAR marker (Souframanien and Gopalakrishna 2006). Similarly, an F_2 population was developed from a cross of DPU 88-31 (resistant) and AKU 9904 (susceptible) cultivars of *V. mungo.* Later, a bulk segregant analysis (BSA) was performed in this F_2 population. Consequently, an SSR marker (CEDG180) was located nearly 13 cM distant from the MYMIV-resistance gene (Gupta and Gopalakrishna 2013). In another study, Maiti et al. (2011) designed degenerate primers related to the consensus motifs of NBS domains from MYMIV-resistance genes in the Fabaceae family. As a result, two MYMIV resistance-specific RGA markers (YR4, CRY1) were identified. Additionally, the CRY1 marker was effectively used in distinguishing resistant plants in F_2 and lateral generations. In *V. mungo*, Souframanien et al. (2010) identified QTLs associated with bruchid beetle resistance in five different linkage groups (LGs) with 8–16% effects on phenotype (Table 2.3).

Functional genomics is enabling researchers to make use of genomic and transcriptomic data to study functions and interactions of genes or proteins. The following sections describe the use of functional genomics strategies (Fig. 2.2) for MAS breeding in *Vigna mungo.*

Table 2.3 Molecular mapping of agronomically important traits in *Vigna mungo*

Trait name	QTL/Genes	Markers linked	References
Twisted and curly leaf	LG8	SSR, RFLP, AFLP	Chaitieng et al. (2006)
Mung bean yellow mosaic virus	MYMV resistance locus	RGA	Basak et al. (2004) and Maiti et al. (2011)
		ISSR, SCAR	Souframanien and Gopalakrishna (2006)
Bruchid beetle resistance	QTL (LG1, LG2, LG3, LG4 and LG10)	RAPD, FLP, SSR, ISSR	Souframanien et al. (2010)
Mung bean yellow mosaic India virus	Gene closely linked to CEDG marker	SSR	Gupta and Gopalakrishna (2013)
Bruchid beetle resistance	qVmunBr6.1 and qVmunBr6.2	SNPs	Somta et al. (2019)

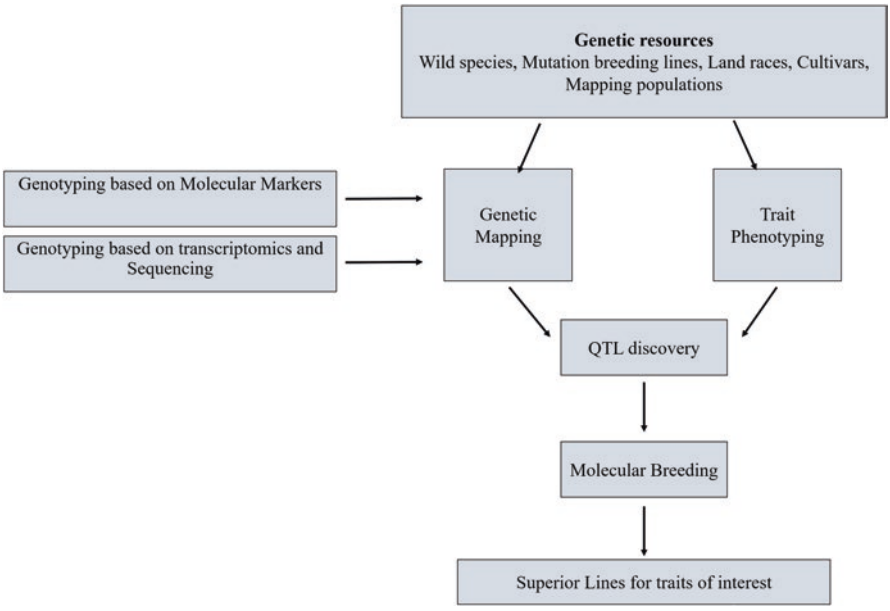

Fig. 2.2 An overview of potentially coordinated research activities on development and application of conventional and functional genomics resources for the improvement of *Vigna mungo*

2.5.1 MAS for Mung Bean Yellow Mosaic India Virus (MYMIV)

Next-generation sequencing (NGS) technology equipped with larger throughput genotyping is readily evolving. These approaches have recently been used for genetic improvement of previously ignored crops. Souframanien and Reddy (2015) performed transcriptomic sequencing of the premature seed of *Vigna mungo* by

Illumina paired-end sequencing to produce 17.2 million paired ends, which represented 33.766 transcript contigs and effectively identified 933 SSR loci. These SSR markers defined a valuable source of marker-assisted selection, comparative genomics, linkage mapping and genetic diversity in black gram. Black gram is extremely prone to mung bean yellow mosaic India virus (MYMIV), which reduces crop yield. In order to understand the *V. mungo* molecular defense mechanism, a MYMIV-resistance gene was introduced to a resistant cultivar (VM84) and a susceptible cultivar (T9) (Basak et al. 2004; Kundu and Pal 2012). Later, high-throughput screening (HTC) was performed for resistant genotypes, which helped in the identification of 13 novel, 8 nonconserved and 45 conserved miRNAs (Paul and Kundu 2014). An earlier study provided initial molecular insights on transcript modulations in *V. mungo* upon MYMIV infection (Paul and Kundu 2014). Nevertheless, HTC profiles of miRNA expression during MYMIV-plant interaction remain unexplored. Kundu et al. (2017) explored the expression of pathogen-responsive miRNA by using a NGS approach combined with an illumine platform. It was observed that after the inoculation of MYMIV, a differential expression was detected for miRNAs belonging to miR156, miR159, miR160, miR166, miR398, miR1511, miR1514 and miR2118 families, and some novel miRNAs detected from the vmu-miRn7, vmu-miRn8, vmu-miRn13 and vmu-miRn14 families. Recently, Choudhary et al. (2018) reported the identification of long noncoding RNAs (lncRNAs) in *V. mungo*. Such studies will definitely provide large-scale and low-cost screening of segregating individuals to screen for desirable phenotypes (Nadeem et al. 2018). It will make marker-assisted selection cheaper and more effective and useful in future.

2.5.2 *Genotyping by Sequencing in* Vigna mungo

Marker-assisted selection (MAS) based molecular breeding depends upon DNA markers that are strongly linked to traits of interest (TOI). Consequently, in MAS, high-resolution trait mapping is required to identify markers highly linked with TOI. Similarly, such high-resolutions mapping strategies are also useful to categorize relevant genomic regions or candidate genes (Table 2.3). Currently, a number of advanced sequencing techniques are readily available. This has enabled researchers to easily identify numerous insertions/deletions (indels) and nucleotide polymorphisms (SNPs) in an organism at very low cost. Various genotyping procedures have been established that are entirely based on sequencing. These methods include restriction-associated DNA sequencing (RAD-seq) (Baird et al. 2008), genotyping-by-sequencing (GBS) (Elshire et al. 2011), diversity array technology sequencing (DArTseq) (Cruz et al. 2013) and specific-locus amplified fragment sequencing (SLAF-seq) (Sun et al. 2013). Somta et al. (2019) established a high-density linkage map for *Vigna mungo* using the SLAF-seq technique and identified two QTLs (*qVmunBr6.1* and *qVmunBr6.2)* for seed resistance to the beetle *Callosobruchus maculatus*.

2.6 Genetic Engineering

Conventional breeding methods have long been employed for crop improvement. However, constraints like the length of time necessary for developing a new cultivar, or difficulties in transferring a trait of interest, have convinced researchers to develop novel strategies. Genetic engineering is a proven and efficient approach to developing cultivars with improved traits in a relatively short period of time. However, it is difficult to use black gram for in vitro culture and genetic transformation. Bhargava and Smigocki (1994) used germinated embryos of *Vigna mungo* as explants in an attempt at genetic transformation through the particle bombardment method. Only a very few studies have tried *Agrobacterium*-mediated transformation in *V. mungo* by employing shoot apex (Saini et al. 2003) and cotyledonary nodes (Bhalla-Sarin et al. 2004; Bhomkar et al. 2008; Chopra and Saini 2014; Muruganantham et al. 2007; Saini and Jaiwal 2002; Saini et al. 2003; Varalaxmi et al. 2013) as explants. Although a few such studies have given encouraging results, there remain numerous limitations for genetic transformation of *V. mungo*. The critical features for efficient transformation include mechanical wounding of explants, time interval for regeneration and a second round of selection on antibiotics at rooting stage. However, the lower transformation efficiency is also attributable to an inefficient T-DNA delivery, existence of inadequate regeneratable cells and short-lived regeneration capacity of cells (Saini and Jaiwal 2005).

Recently, Kapildev et al. (2016) developed a much more efficient and improved method of *Agrobacterium*-mediated transformation of recalcitrant black gram cv. T9 (Fig. 2.3). Although there are some success stories of *Vigna mungo* genetic engineering for improved traits (Table 2.4), it is anticipated that further improvements in *V. mungo* stable genetic transformation may contribute significantly to enhance *V. mungo* abiotic/biotic stress resistance and nutrition.

2.7 Transfer of *Vigna mungo* Genome into *V. radiata* Through MAS-Based Introgression

Low concentration of certain amino acids, like methionine, valine, tyrosine, threonine, leucine, isoleucine and cysteine is a limiting factor in *Vigna radiata* quality. In *V. mungo*, the concentration of amino acids like methionine (21.33%), cysteine (5.5), isoleucine (20.83), leucine (7), threonine (5.85), tyrosine (3.7) and valine (6.19%) are higher as compared to *V. radiata* (Zia ul Haq et al. 2014). Genetic improvement in *V. radiata* was attempted through interspecific hybridization with *V. mungo* mostly for disease resistance (Bhanu et al. 2017a, b; Pandiyan et al. 2010; Sehrawat et al. 2016; Singh et al. 1996, 1997, 2006). Singh (1990) reviewed various crossability studies among *Vigna* species and suggested that *V. radiata* may produce effective hybrids when used as a female parent with *V. umbellate, V. angularis* and *V. mungo*. Although the reciprocal crosses were nonviable, the embryo rescue

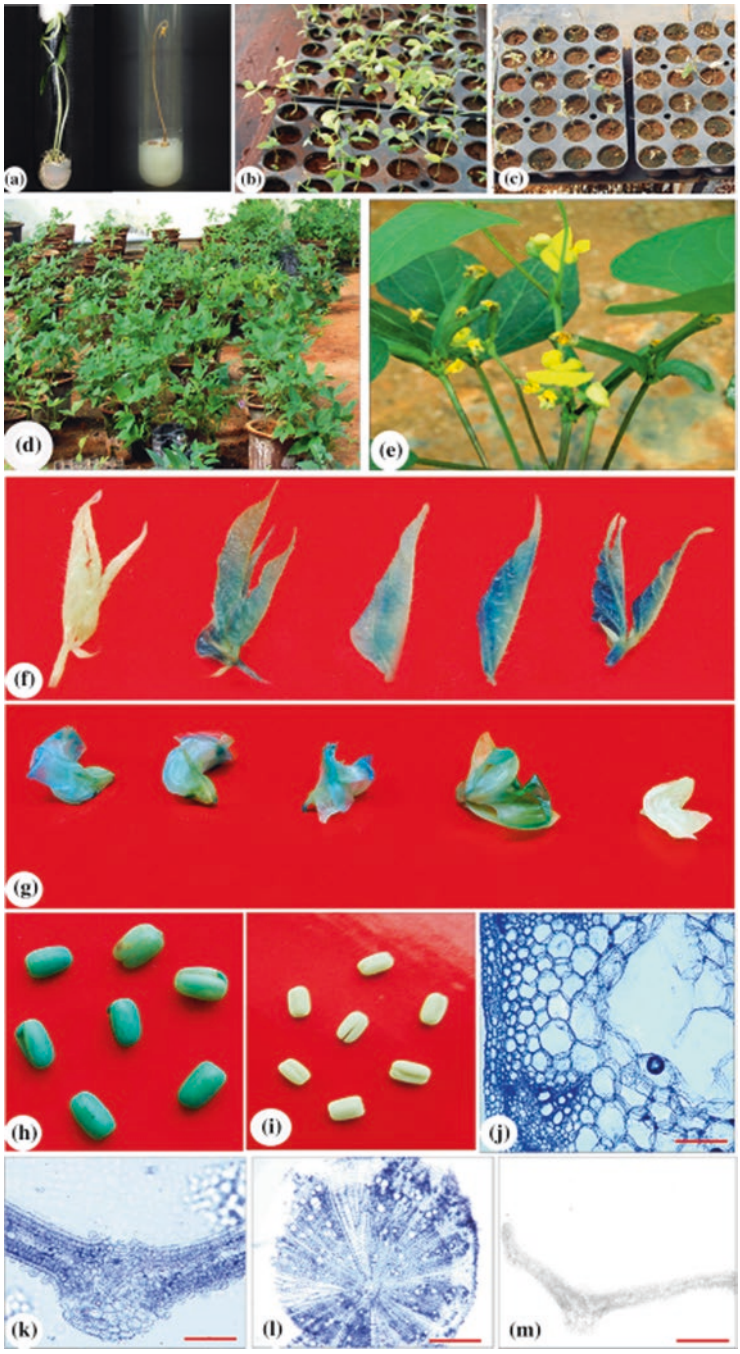

Fig. 2.3 *Agrobacterium-mediated* in planta *transformation of black gram cv. T9.* (**a**) Transformed and nontransformed plants in MS selection media, (**b**) Root trainers, (**c**) 15-day-old transformed and nontransformed plants on selection media, (**d**) Plants in greenhouse, (**e**) Flowers and pods, (**f**) Leaves (**g**) GUS staining in flowers, (**h**) GUS staining in seeds, (**i**) Nontransgenic seeds. (**j**) GUS expression in root cross-sections, (**k**) GUS staining in leaf cross-section, (**l**) GUS expression in stem cross-section, (**m**) Nontransgenic leaf. (Source: Kapildev et al. 2016)

Table 2.4 Genetic engineering of *Vigna mungo* by stable transformation methods

Feature	Gene of interest	Trait	References
Abiotic stress tolerance	Expression of *glyoxalase I* gene using a novel cestrum yellow leaf curling virus promoter	Salt stress alleviation	Bhomkar et al. (2008)
	Overexpression of *ALDRXV4* gene via reactive carbonyl detoxification	Multiple stress tolerance (drought, salt, methyl viologen and H_2O_2)	Choudhary et al. (2018)
Disease resistances	Barley chitinase and ribosome-inactivating protein genes	Resistance to C*orynespora* leaf spot fungal disease	Chopra and Saini (2014)
	Bacterial chitinase (*ChiB*) gene	Resistance against E*rysiphae polygoni*, induced powdery mildew disease	Das (2018)

technique has been proven useful in making interspecific hybrids for the reciprocal crosses (for details see: Pratap et al. 2018). However, *V. mungo* and *V. radiata* reciprocal hybrids could be successfully obtained using the sequential embryo rescue technique (Verma and Singh 1986). Superior high yielding and disease resistant *V. radiata* × *V. mungo* recombinants having higher concentration of essential and non-essential amino acids were identified and the inheritance of gene(s) responsible for improvement was also studied (Abbas et al. 2016).

Molecular confirmation of interspecific recombinants is essential to overcome issues like self-pollination, environmental influence and inadequacy of morphological characteristics during interspecific hybridization (Abbas et al. 2015). The DNA based marker assisted approach has provided evidence for genetic confirmation of *Vigna radiata* and *V. mungo* interspecific recombinants (Table 2.5) and escalated the authenticity of selection in the *V. radiata* improvement program. Different marker systems like URP, RAPD and SSR have been used for genetic differentiation of *Vigna* species and it was concluded that the SSR marker system was more efficient in detecting genetic variability among all the *Vigna* species (Abbas et al. 2015; Dikshit et al. 2007). Recently, a unique process was developed for the genetic improvement of *V. radiata* nutritional quality through recombination with *V. mungo* (Abbas et al. 2019). Backcrossing of *V. radiata* × *V. mungo* recombinants with *V. mungo* parents yielded a plant structure resembling *V. mungo* as shown in Fig. 2.4.

2.8 Web Resources for *Vigna mungo*

Black gram is grown in many tropical and subtropical areas of Asia. It is an important source of nutritional elements, dietary proteins and it also plays a role in immune response of human for its medicinal properties. Mung bean yellow mosaic India virus (MYMIV) causes yellow mosaic disease, which causes significant losses of crop in terms of yield. Genotypes with contrasting traits are important sources for

Table 2.5 Details of polymorphic RAPD, RIS and SSR primers used for molecular confirmation of *Vigna radiata* × *V. mungo* interspecific recombinants

Primer code	Sequence (5′-3′)
Random amplified polymorphic DNA (RAPD)	
OPU-3	CTATGCCGA
OPAJ-20	ACACGTGGTC
OPS-07	TCCGATGCTG
Simple sequence repeats (SSR-RIS)	
RIS-F	TAATTTCTGCTTGCTCCATGC
RIS-R	ACTGGGGTGCACTGGATTAG
Simple sequence repeats (SSR)	
VR040	(F) TGACAACATGGGAAGAAGAAGA (R) ACACCAACACAAAAGCAAACAC
VR062	(F) CGAAGACGAAATCTGAAGACAA (R) TTACTTCTCCCAGCACTCCAAT
VR0111	(F) TGCATCTTTATTGAGTTCCGTG (R) GTTTTGGGGTGAATGTTGGATA
VR0304	(F) GAAGCGAAGAAGCCATAGAAAA (R) CCTCACACACAACACAACAGAA

studying the defense mechanism of plants and respective genes. The whole genome sequencing of *Vigna mungo* is not yet completed. Furthermore, genomic resources of this crop are not freely accessible. Hence, a transcriptome database of *V. mungo* was established using two contrasting varieties viz., cv. VM84 (resistant) and cv. T9 (susceptible). The database is available at www.webtom.cabgrid.res.in/vmtdb/ (Jasrotia et al. 2017). De novo assembly was established using CAP3 and Trinity. Among the 240,945 unigenes, 68.8% (165,894) displayed similarity with already known genes of the NR database and 31.2% were identified as novel genes. In same study, 22,101 genes were found to be differentially expressed among all datasets. Jasrotia et al. (2017) also identified 4105 SNPs and indels, 44,335 putative genic SSR markers, 64,964 transcriptional factors and 137 pathways. Salicylic acid-binding protein 2-like, MAPK, NBS-LRR and pathogenesis-related protein domains were also identified, which play a vital role in defense mechanisms against pathogens. It is the first web resource of black gram for future genome annotation and readily available molecular markers for crop improvement (Jasrotia et al. 2017).

The International Atomic Energy Agency (IAEA), along with its partner organizations, is promoting mutation breeding for sustainable food security in all parts of the world, and specifically by decreasing the vulnerabilities of food production systems under climate change. In this context, FAO and IAEA maintain the FAO/IAEA Mutant Variety Database (MVD) (https://mvd.iaea.org/) to collect information on plant mutant varieties (cultivars) globally released on an official or commercial scale. There are records of nine *V. mungo* mutant varieties in this database.

The *Vig* GS is another database for the *Vigna* species, which comprises genome data and is an important development on the way to the comprehensive understanding of plant adaption under stress conditions (Sakai et al. 2015). Presently 13 wild

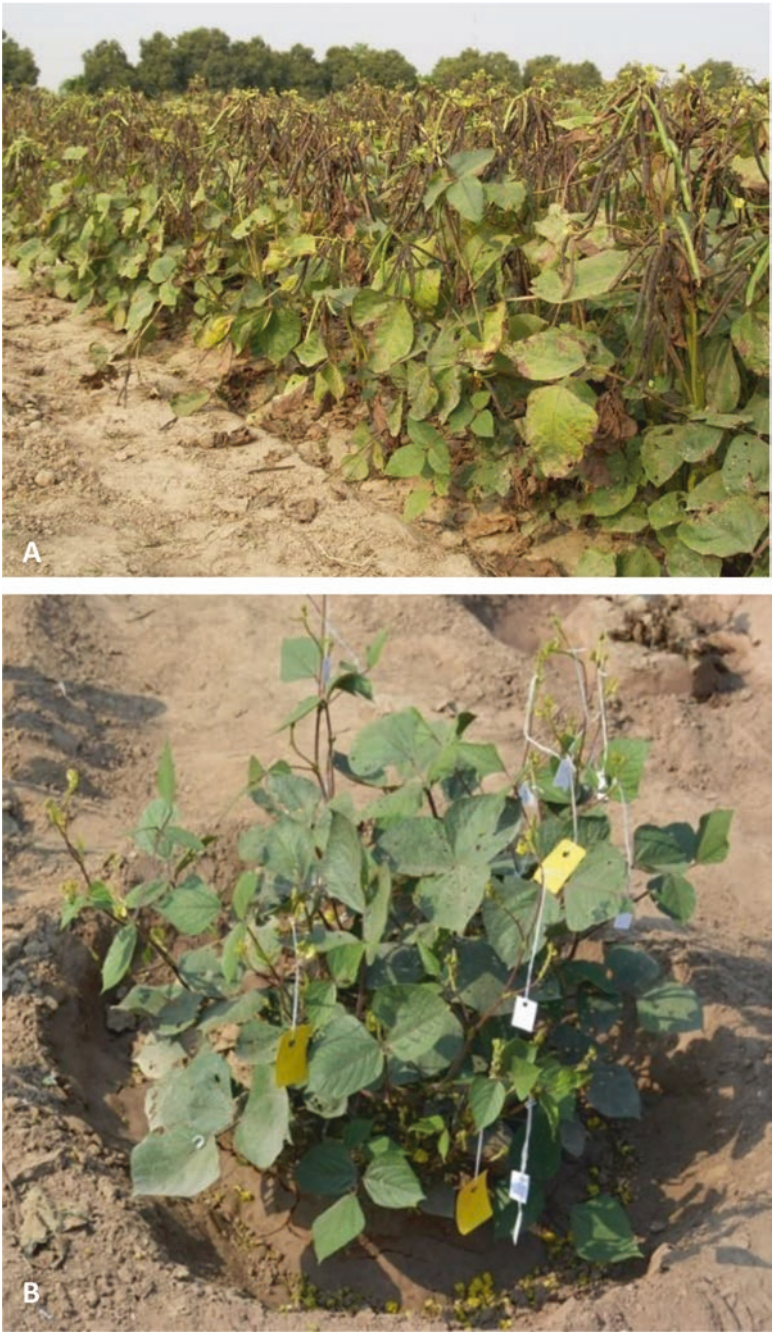

Fig. 2.4 MAS based introgression between *Vigna mungo* and *V. radiata* developed by the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. (**a**) Superior genotype developed through recombination with *V. mungo*, (**b**) Interspecific recombinant plant with *V. mungo*-type structure

Table 2.6 List of wild *Vigna* species currently in use for whole genome sequencing

Subgenus	Section	Species
Ceratotropis	*Aconitifoliae*	*V. aconitifolia* (Jacq.) Maréchal
		V. aridicola N. Tomooka & Maxted
		V. indica T.M. Dixit, K.V. Bhat & S.R. Yadav
		V. stipulacea Kuntze
		V. trilobata (L.) Verdcourt
	Ceratotropis	*V. mungo* (L.) Hepper
	Angulares	*V. exilis* Tateishi & Maxted
		V. minima (Roxb.) Ohwi & Ohashi
		V. nakashimae (Ohwi) Ohwi & Ohashi
		V. riukiuensis (Ohwi) Ohwi & Ohashi
Plectrotropis	*Plectrotropis*	*V. vexillata* (L.) A. Rich.
Vigna	*Vigna*	*V. luteola* (Jacq.) Benth
		V. marina (Burm.) Merr.

Vigna species are employed for genome sequencing (Table 2.6). After the complete annotation of these genomes, sequences of these wild species will be used for comparative protein mapping, based on individual genome or alignment of genomes of closely related *Vigna* species. Data would be executed in *Vig* GS. Data are expected to offer basic information on the loss and gain of genes and rearrangement of genomes (Sakai et al. 2015).

2.9 Conclusions and Future Perspectives

Although perceptible progress has been accomplished to understand *Vigna* genomics, there is still a lot to do for *V. mungo*. Many *Vigna* species possess a narrow genetic background that limits the polymorphism of markers within the cultivated germplasm. Because of this limitation, most genetic linkage maps of *Vigna* species have been developed using interspecific and intersubspecific crosses. These maps have reduced utility in breeding programs for exploiting the genetic variation of the cultivated genepool. Furthermore, the number of markers mapped on many of these genetic maps are of low to moderate density. Therefore, there is a need to develop dense genetic linkage maps in *Vigna* species (especially *V. mungo*) based on intraspecific mapping populations.

To construct genetic maps along with multi-site phenotyping for traits of interest, a variety of genetic resources can be utilized by means of different genotyping platforms. Transcriptomic and genomic resources such as ESTs, genes, molecular markers, genome sequences and transcriptome assemblies are developed by using conservative and next-generation sequencing (NGS) technology platforms. Genotypic and phenotypic data can be used to identify QTLs/markers related with target traits utilizing QTL mapping or association mapping applications. In modern

breeding programs, QTL and genetic information is used for marker-assisted backcross breeding (MABS). By utilizing these modern breeding applications, superior lines for desired traits are generated enhance crop productivity.

Limited genomic resources are the main hurdle in the improvement of *Vigna mungo*. Although efforts have been made to develop molecular markers, the number of molecular markers available is still very low. With reductions in the cost of sequencing, an increasing number of genome and transcriptome sequencing techniques are routinely becoming available. Therefore, efforts should be made to develop improved molecular markers in this crop and utilize them for molecular breeding.

The use of high throughput marker genotyping techniques must provide the required stimulus to expand the genomic resources in *Vigna mungo*. Further, the whole genome of the Korean mung bean cv. Sunwhanokdu has been sequenced and de novo assembled into contigs and scaffolds. In addition, completed whole genome sequences of soybean, alfalfa and lotus (*Lotus japonicas* L.) will provide a vast genomic resource for comparing the genomes and transferring the information from these model legumes to *Vigna* species. This would also help in the fine mapping and cloning of genes and QTLs for agronomically-important traits and in the breeding of elite cultivars resistant to biotic and abiotic stresses through MAS.

It is very likely that future *Vigna mungo* production will be affected by a number of climate change elements including higher temperature, drought and elevated CO_2 levels. Subsequently, these plants are expected to experience faster developmental rates, shorter growing seasons, reduced duration of terminal drought, decreased photosynthetic efficiency, augmented rate of flower and pod abortion, defective pollination, progressive decline of seed quality and increased carbon gain favored by elevated atmospheric CO_2. Additionally, regional yield variations will also be influenced by native manifestation or interaction of climate change factors. Such predictions specify the need of extraordinary measures for substantial improvements in productivity of this crop. Nevertheless, there is a considerable lack of tools for genetic characterization or improvement of *V. mungo* i.e. mutagenesis, reverse genetics, stable and transient gene silencing or TILLING platforms. Alternative novel technologies of targeted mutagenesis and genome editing (CRISPR/Cas9 system) could facilitate prompt improvements of this crop. Already, CRISPR/Cas9 has been used to study symbiotic nitrogen fixation (SNF) in legumes, which is expected to enhance our knowledge of legume-rhizobia interactions and engineering of the SNF pathway into nonlegumes. In conclusion, impending advances in legumes (especially ignored crops like *V. mungo*) for sustainable agriculture will require integration of several fields of physiology, crop management, genetics, biotechnology, conventional and nonconventional breeding.

Appendices

Appendix I: Major Institutes Engaged in Research on Vigna mungo

Country	Name of institute	Website	Number of accessions
Bangladesh	Bangladesh Agricultural Research Institute (BARI)	http://www.bari.gov.bd	339
	Bangladesh Agricultural Research Council (BARC)	http://www.barc.gov.bd/	106
Colombia	Centro de Investigación La Selva, (CoRPOICA) (now AGROSAVIA) Rionegro Antioquia	http://www.corpoica.org.co	108
India	ICAR-National Bureau of Plant Genetic Resources (NBPGR)	www.nbpgr.ernet.in	3131
	Indian Agricultural Research Institute (IARI)	www.iari.res.in	90
	Bhabha Agriculture Research Centre	https://www.barc.gov.in	–
	Indian Council of Agriculture Research Institute, Tamil Nadu	http://www.icar.org.in	113
Nepal	Nepal Agricultural Research Council (NARC)	http://narc.gov.np/	83
Pakistan	Pakistan Agriculture Research Council, PGRI/NARC, Islamabad	http://www.parc.gov.pk	693
	Ayub Agriculture Research Institute (AARI), Faisalabad	https://aari.punjab.gov.pk	50
	Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad	http://www.niab.org.pk	112
Russian Federation	Vavilov Institute of Plant Genetic Resources (VIR)	https://www.vir.nw.ru/en	210
Taiwan	The World Vegetable Center	https://avrdc.org/	481
USA	Southern Regional Plant Introduction Station, USDA-ARS, Griffin, GA	https://www.ars-grin.gov	300
Japan	The National Institute of Agro-biological Sciences (NIAS)	http://www.naro.affrc.go.jp	1198
China	National Crop Germplasm Resources Platform	http://www.cgris.net	469
	Yunnan Academy of Agriculture Sciences	http://www.yaas.org.cn	300
Belgium	Walloon Pulses Research Centre, Gembloux	http://www.cra.wallonie.be	79

Appendix II: Important Cultivars and Accessions of V. mungo

Cultivar/ accessions	Important traits	Developer/Maintainer institute
EC319031-33	High yield, flood tolerance	ICAR-NBPGR, India
EC319034-37	Drought tolerance	
IC553269	Brown pod and yellow seed	SVBPUA&T, Meerut, India
IC296878	Dwarf semi-erect with ground pod bearing habit	CCSHAU, Hisar, INDIA
VBG-09-012	Multipod formation at base of peduncle, leaf axils and base of clusters	NPRC, Vamban, Pudukkottai, Tamil Nadu, India
VBG-04-014	Unique plant type	
PU 07-7	Large seeded, mung bean yellow mosaic virus (MYMV) resistant	Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India
PU 08-1	Large seeded, early maturing, MYMV resistant	
PU 08-4	Early maturing, MYMV resistant, high yield/plant	
PU 06-16	Early maturing, higher pod length, MYMV resistant	
PMU 01	Susceptible to MYMV, higher seeds/pod and pods/plant	
Pant U-31	Early, dwarf and compact plant type, resistant to MYMV, released for commercial cultivation	
Pant U-40	Erect plant type, resistant to MYMV, released for commercial cultivation	
M-01001-1 and M-6036-21	Drought tolerance	Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan
M-97 and Arroj-II	Drought susceptible	
MASH 97	Semi-erect growth habit, early maturing and tolerant against lodging	Pulses Research Institute, (AARI), Faisalabad, Pakistan
MASH 2	Semi erect, medium early maturing, high yielding, low shattering	NARC, Islamabad, Pakistan
MASH 3	Erect growing, medium early maturing	
CHAKWAL MASH	Semi-erect, short duration, for arid agriculture regions	BARI, Ckakwal, Pakistan
MASH 88	Semi-erect, medium long maturing	Pulses Research Institute, (AARI), Faisalabad, Pakistan

(continued)

Cultivar/ accessions	Important traits	Developer/Maintainer institute
BARI Mash-1	A medium statured (45–50 cm), semi erect cultivar, tolerant to yellow mosaic virus	Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh
BARI Mash-2 (Sarath)	BARI Mash-2 is erect and attains a height of 33–35 cm. Tolerant to yellow mosaic virus	
BARI MASH-3 (HEMANTA)	Erect growth habit and attains a height of 35–38 cm, high yielding, tolerant to yellow mosaic virus	
BARI Mash-4	Dwarf plant type, tolerant to yellow mosaic virus	
Phitsanulok 2	Better sprouting and nutrition	Chai Nat Field Crops Research Center, Chai Nat, Thailand
Chai Nat 80		
Uthong 2		
MI – 1	Susceptible to mung bean yellow mosaic virus (MYMV), *Cercospora* leaf spot and bruchids	Field crop research and development institute, Department of Agriculture, Sri Lanka

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Chapter 3
Chickpea (*Cicer arietinum* L.) Cytogenetics, Genetic Diversity and Breeding

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Abstract Climate change, depleting natural resources, declining arable land and sky-high population represent the main obstacles to the attainment of global food security. Therefore, to make a significant breakthrough in the food production and to combat global food insecurity, sustainable intensification of the agricultural production through low-input agriculture and development of cultivar with improved yield and adaptability is required. By traditional and modern plant breeding methods, breeding of pulses, cereals, and other important food crops, especially chickpea, can be accomplished by exploiting available genetic diversity. Chickpea and other pulse crops are important foods in many nations and play a vital role in the diet of malnourished populations world wide. Globally, chickpea is mainly grown in developing countries, accounting for ~97% of world area and 96% of world production. At present the average global yield of chickpea is 0.9 mt/ha, very low comparedto its estimated potential of 6 mt/ha under favorable growthconditions. The main constraints that limit desired goals of chick peaproductivity include low genetic variability, low and unstable yield and low resistance to biotic and abiotic

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stresses. Chickpea being a self-pollinated crop harbors low genetic variability. Mutation breeding is the logical tool to create variability in a crop species in a very short span of time, as compared to breeding methods. This chapter cover sorigin, classification, cytogenetics, germplasm and breeding methods for chickpea improvement.

Keywords Breeding approaches · Chickpea · Genetic variability · Marker assisted · Mutation breeding · Production constraints

3.1 Introduction

Pulse crops play avital role in curbing and reducing food insecurity and malnutrition problems in developing countries like India, Pakistan and Bangladesh. The decline in per capita availability of food due to an unparalleled rate of population growth gravely demands the attainment of enhanced productivity, especially in developing countries. According to the Food and Agriculture Organization of the United Nations (FAO), human population will increase to 9 billion by the end of 2050 and the feeding of such a huge population will be a challenging task. With the skyrocketing population growth, dwindling arable lands, depleting water resources, increased urbanization and industrialization, hunger is a ghost potentially haunting millions of poor people across the globe.

The erratic climate change, abiotic and biotic stresses influence significantly the production and yield are additional obstacles to attain the goal of global food security (Ahmad et al. 2019a, b; Naikoo et al. 2019). In the current scenario, food production cannot be increased by further exploitation of natural resources such as land and water; the only way to enhance production is through the establishment of low-input agriculture in the twenty-first century. Low input agriculture is based on the development and release of crop cultivars which are climate-change resilient, genetically diverse, input use-efficient, high yielding and widely adaptable to a range of agro-ecosystems (Mba 2013). Therefore, to make a significant breakthrough in the food production and to combat the global food insecurity, sustainable intensification of agricultural production through low-input agriculture and development of cultivars with improved yield and adaptability is required.

Breeding of pulses, cereals and other important food crops, especially chickpea, by exploiting available genetic diversity using traditional methods such as mass selection, pedigree selection and hybridization has been practiced in the past. Nonetheless, these methods in the current era are inadequate to make any significant contribution to manage the world's rapidly growing food demand. The available genetic variability in food crops, particularly grain legumes, has been exhausted, which necessitates the induction of innovative breeding tools for generating new genetic variability in the yield traits. In this bizarre scenario, induced mutagenesis has emerged as a potential remedy, largely exploited for enhancing the genetic variability. This can unleash new alleles that govern the vital agronomical traits desired for climate-smart agriculture, *smart crop* cultivars, for the twenty-first century. Chickpea, being a nutritious grain legume and climate resilient, fits well in the tar-

geted food crops to be improved into the genre of *smart crops* and hence, appropriate scientific investment in the chickpea genetic improvement is required. Kozgar et al. (2014) reported Asian chickpea germplasm, being self-pollinated, harbors little genetic variability, which raises concern over the reduction in yield due to biotic and abiotic stresses. Erskine et al. (1998) and Toker et al. (2007) recommended mutation breeding as an extremely important technique to broaden the genetic base, while reviewing the different plant breeding methods utilized in various self-pollinated crops. During the past nine decades, around 3300 improved crop cultivars in about 210 plant species have been developed by induced mutations and officially released across the 60 nations (http://mvgs.iaea.org). A huge number of mutant cultivars in a wide range of crops have been developed and officially recommended for cultivation in different developing nations and have provided huge monetary gains. Recently, mutagenesis has received a gigantic boost for its use in an innovative promising technique known as targeting induced local lesions in genomes (TILLING).

Chickpea, also known as Bengal gram, is a grain legume cultivated by poor farmers in almost all parts of world, including Asia, Africa, Europe, North America and South America. Globally 13.2 million ha of cultivated land is devoted to chickpea cultivation, producing approximately 11.62 million mt annually. India contributes about 67.7 % to the world's total production and is the top chickpea producing nation (FAO 2016). Chickpea is an annual, self-pollinated, winter season crop and is the second most important food legume crop after common bean. Chickpea seeds are nutrient rich and contain adequate amounts of carbohydrates, proteins and fats, and offer a good replacement for meat, especially among malnourished populations. Chickpea is used to make flour, and it is also consumed in several forms, roasted as a snack, raw, and the dry seeds are used for making soups and porridge. The objective of this chapter is to cover the origin, classification, cytogenetics, germplasm and breeding methods for chickpea improvement.

3.1.1 History, Origin and Domestication

The genus *Cicer* belongs to the family Leguminosae, subfamily Papilionoideae and the monogeneric tribe Cicereae Alef. However, some scientists argue that it belongs to tribe Vicieae Bronn (Singh et al. 1997a, b). The genus is comprised of 35 perennial, 8 annual and 1 unspecified wild species (Toker 2009; Van der Maesen et al. 2007). On the basis of morphological traits and geographical distribution the tribe Cicereae has been further sub-classified into sections *Cicer*, *Chamaecicer*, *Polycicer* and *Acanthocicer* (Van der Maesen et al. 2007). The single cultivated species *Cicer arietinum* belongs to the section *Cicer*. Harlan and de Wet (1971) proposed the primary and secondary gene pools of wild chickpea on the basis of crossability with chickpea, which inturn reflects their distance from the cultivated species. The wild annual progenitor *C. reticulatum* Ladz. and the closely related *C. echinospermum* P. H. Davis represent the primary gene pool, while the secondary gene pool is composed of *C. bijugum* K. H. Rech, *C. pinnatifidum* Jaup and Spach and *C. judaicum* Boiss.

Ramanujam (1976) is of opinion that Turkey and adjoining areas of Iraq, Iran and the former Soviet Union represent the center of origin of the cultivated chickpea. Different researchers have reported different origins of chickpea such as the southern Caucasus, northern Persia (Iran) (De Candolle 1883) and the Mediterranean, Central Asian and Indian regions (Vavilov 1926). It is presumed that large-seeded chickpea reached India via the Afghan capital of Kabul and acquired the name in Hindi as *Kabuli Chana* about two centuries ago (Van der Maesen 1972, 1987). The most primitive record of chickpea in India is from 2000 BC, from Atranji Khera in Uttar Pradesh (Chowdhury et al. 1970). It was introduced in Peninsular India probably between 500 and 300 BC (Vishnu-Mitre 1974).

Initially chickpea was domesticated in a southeast region of Turkey in association with crops like wheat, rye, barley, lentil, pea, vetch and flax (Abbo et al. 2003; Ladizinsky and Adler 1976). The oldest records of chickpea used as food are: eight millennium BC at Tell el-Kerkh (Tanno and Willcox 2006) and Tell Abu Hureyra Syria (Hillman 1975); 7500–6800 BC at Cayonu Turkey (Van Zeist and Bakker-Heeres 1982) and 5450 BC at Hacilar Turkey (Van der Maesen 1984). The earliest date for cultivation of chickpea is the remains at Tell el-Kerkh where both *Cicer arietinum* and its progenitor *C. reticulatum* were clearly distinguished, but no such distinction was possible from the remains at Tell Abu Hureyra, Syria (Tanno and Willcox 2006). Archeological records of *Cicer* domestication are meager as in carbonized seed the distinguishing seed beak gets broken off, but its cultivation is well known in Egypt and the Middle East from 3300 BC onwards (Van der Maesen 1972). Based on the current distribution of the wild progenitor chickpea, *C. reticulatum*, and early Neolithic chickpea in the Fertile Crescent, mainly in southeast Anatolia, it can be concluded that chickpea is likely to have been domesticated in southeast Anatolia (Ladizinsky and Adler 1976). The world distribution map of kabuli, desi, pea-shaped and wild chickpea is presented in Fig. 3.1. Moreno and

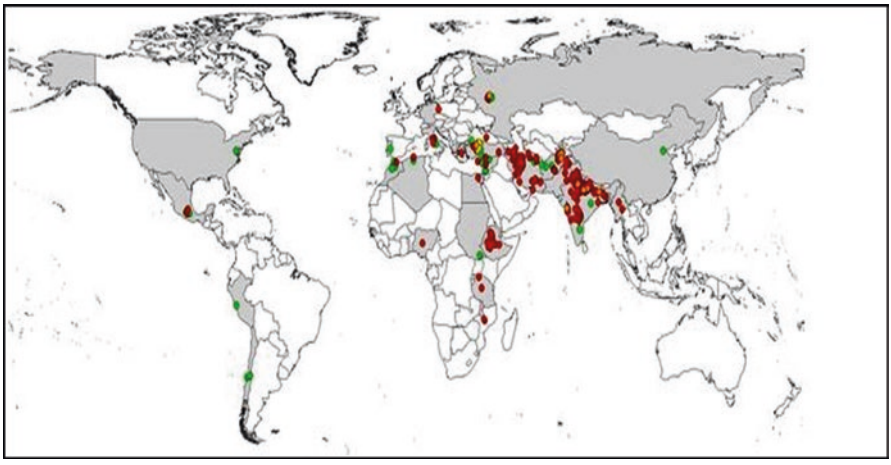

Fig. 3.1 The chickpea distribution, kabuli in green, desi in red, pea-shaped in orange and wild in yellow color dots. (Source: Thudi et al. 2014)

Cubero (1978) are of the view that the domestication seems to have occurred from the wild progenitor *C. reticulatum* (syn. *C. arietinum*ssp. *reticulatum*) with monophyletic origin, as shown by the low genetic variation of the cultigen *C. arietinum* ssp. *arietinum*.

3.1.2 Botanical Description and Classification

Chickpea, a member of the subfamily Fabaceae (syn. Papilionaceae) is a short annual herb attaining a height of around 1 m. The growth habit varies from erect, semi-erect, spreading, semi-spreading and prostrate. The plant possesses a deep taproot with several lateral roots and comprising a robust root system. The stem is erect, pubescent with three types of branching viz. primary, secondary and tertiary, and branching from the base at ground level which imparts to the plant a bushy appearance. Leaves are imparipinnately compound with 6–8 pairs of hairy leaflets arranged in an opposite or alternate manner on a rachis with a small petiole. The shape of leaflets is elliptic or oval with serrated leaf margins. Flowers are borne singly in axillary racemes and are pedicellate, bisexual with papilionaceous corolla and diadelphous stamens (9 + 1). The ovary is unicarpellary, unilocular and superior harboring 1–2 ovules. The fruit is an inflated pod which is pubescent, covered with glandular and non-glandular hairs bearing 1–2 seeds which may have either a smooth or wrinkled seed coat. Germination is hypogeal and the seeds of cultivated chickpea do not exhibit any dormancy period (Khan et al. 2011). Based upon seed size and color, chickpea is categorized as *macrosperma* or *kabuli* type and *microsperma* or *desi* type.

Macrosperma or kabuli type: Seeds are bold, large and weigh around 0.3–0.5 g each, seed coat is thin ranging in color from white to pale cream. The plants do not possess anthocyanin pigmentation. It is believed that the desi type cultivars have undergone natural mutation and selection to give rise to kabuli type cultivars (Gil and Cubero 1993; Hawtin and Singh 1980; Moreno and Cubero 1978; Salimath et al. 1984). Toker (2009) is of opinion that spontaneous mutations in *Cicer reticulatum* could be a possible reason for the evolution of kabuli cultivars.

Microsperma or desi type: Seeds are small and weigh around 0.2 g each and are less than one-half the size of kabuli type and have a thicker seed coat ranging in color from brown to yellow.

The genus *Cicer* has 9 annuals and 34 perennial species and is subdivided in to four sections: *Cicer*, *Chamaecicer*, Polycicer and *Acanthocicer* on the basis of criteria that include phenotype, habitat and lifespan (Van der Maesen et al. 2007). Chickpea was included in the tribe Vicieae, but due to its distinctive traits, it was moved in the monogeneric tribe Cicereae (Kupicha 1977). The taxonomy of chickpea is as follows:

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Superdivision:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order:	Fabales
Family:	Fabaceae (Leguminosae)
Subfamily:	Faboideae (Papilionaceae)
Tribe:	Cicereae
Genus:	*Cicer*
Species:	*arietinum*

3.1.3 Cytogenetics

All chickpea cultivars and their wild relatives are self-fertilizing diploids (2n = 2x = 16 chromosomes) (Ahmad and Godward 1980; Mercy et al. 1974; Singh and Singh 1997) with a genome size of 740 Mbp (Varshney et al. 2013b). There are reports of chickpea species with a 2n=14 chromosome number, but presumably they are rare (Singh et al. 1997a, b). Chickpea chromosomes are small and the average length of the mitotic metaphase chromosomes is around 2.2 μm (Ahmad 2000). The structure of chromosomes in chickpea has also been shown to vary in different species and within genotypes of the same species. In both annual and perennial species of *Cicer* an invariant somatic chromosome number exists, yet a huge karyological variation is present. Ahmad (2000) examined nine annual *Cicer* species and noted significant differences in the length of chromosomes and the position of primary and secondary constrictions and concluded that a unified karyotype is not present in all the annual *Cicer* species. These variations in the chromosomal structures have played a role in evolution of cytotypes. In addition to the interspecific karyotype variation, intraspecific karyotypic variation also exits and is supported by several researchers who hold a different opinion with respect to length of chromosome, arm ratio and the secondary constriction position (Ahmad 2000; Ahmad and Hymowitz 1993; Kordi et al. 2006; Ocampo et al. 1992). Ahmad (2000) attributed these differences in opinion to the inconsistencies in the cytological protocols used by researchers.

Secondary constrictions are clearly visible and are formed due to close association of chromosomes with the NOR (nucleolus organizing region). Ohri and Pal (1991), Tayyar et al. (1994) and Kordi et al. (2006) reported that in all species of *Cicer* only one chromosome pair harbors secondary constriction except *C. reticulatum* wherein two pairs show secondary constriction (Ocampo et al. 1992; Ohri and Pal 1991). The conclusion was that during the evolution of *C. echinospermum* and *C. arietinum* from *C. reticulatum*, one of the two NOR loci was lost. Depending on

the species, the position of secondary constriction varies as in the annual *C. arietinum, C. reticulatum* and *C. echinospermum*, the longest chromosome pair harbors it while it is present on intermediate or a small-sized chromosomes in all other species (Ahmad 2000). The variation in the position of the secondary constriction plays an important role in the classification of chickpea as all other chromosomal aspects do not reflect significant variation. According to Ahmad (2000) the length of the mitotic chromosome range is 1.32–3.69 μm and three of the chromosomes are submetacentric and the others metacentric. An insignificant difference in relative chromosome length in the chickpea has been reported as in the kabuli type chickpea; out of the eight chromosomes three are longer than their equivalents in the desi type chickpea, while the other five are longer in desi types but these differences are small, with the range of 0.2–0.8% of the overall relative chromosome length (Ruperao et al. 2014). Kordi et al. (2006) also reported that the similar differences in the chromosome length is less substantial than claimed by Ohri and Pal (1991). Kordi et al. 2006 reported that morphology based recognition of chromosomes in *C. arietinum* are the two chromosomes i.e. shortest metacentric and longest submetacentric chromosome. As per Kordi et al. (2006) only the longest and shortest chromosomes being submetacentric and metacentric are always classified while atleast one of the six remaining chromosomes shows deviation from the mean length and/or arm ratio assigned to the reference accession by Ahmad (2000). Cultivated chickpea harbors more distinctive karyotypes and the differences in the chromosome length are greater than the annual species (Ahmad 2000).

The system of naming chromosome used in *Cicer* varies from author to author, but two main naming systems are commonly used. One is numerical based with longest chromosome assigned as 1 and shortest assigned as 8 (Ocampo et al. 1992), and the other is letter based (A–H), where A = 1… H = 8 (Galasso et al. 1996; Staginnus et al. 1999; Vláčilová et al. 2002; Zatloukalova et al. 2011). Recently, another system was employed based on linkage group numbers (LG) after the first linkage maps were developed. An attempt to cytogenetically characterize the perennial *Cicer* species was carried out by many researchers. In1972, Van der Maesen estimated 2n = 14 or 2n = 16 as the chromosome number in the perennial *Cicer* species. The initial description of the karyotype of the perennial *C. anatolicum* (Ahmad 1989), established 2n = 16 as the chromosome number, as is the case for the annuals. Ensuing analysis revealed much similarity in the karyotype of *C. songaricum* with that of *C. arietinum*, *C. reticulatum* and *C. echinospermum.*

3.1.3.1 Molecular Cytogenetics

The expansion of the fluorescence in situ hybridization (FISH) technique to localize particular DNA sequences on chromosomes has revealed many important features of chromosome organization in many species including *Cicer* species. Abbo et al. (1994) and Staginnus et al. (1999) reported first sequences localized through FISH technique were identified as the ribosomal RNA genes. While only one chromosome pair carries a visible satellite, two sites hybridize with a 45S rDNA sequence,

which was interesting in light of the presence of two satellited chromosome pairs in *C. reticulatum* (Abbo et al. 1994;Ohri and Pal 1991). Additionally, two sites with 5S rRNA and 45S rDNA sequences have been recognised on chromosome B (Vláčilová et al. 2002). About 50% of the chickpea genome is composed of repetitive DNA (Jain et al. 2013; Varshney et al. 2013b). These repetitive sequences are very informative and hence serve as cytogenetic markers, particularly where the chromosomal distribution is nonrandom (Jiang and Bikram 2006; Schwarzacher 2003). The FISH technique led to the detection of five microsatellite motifs {(A)16, (CA)8, (TA)9, (AAC)5, (GATA)4} which were selected based on previous literature (Sharma et al. 1995), but did not produce any chromosome-specific karyotypes. All these five microsatellite motifs are localized within each chromosome but show a varied distribution and intensity from repeat motif to repeat motif (Gortner et al. 1998). Zatloukalova et al. (2011) and Staginnus et al. (1999) reported a site in the pericentromeric region of chromosome A and a major cluster on the short arm of chromosome B. Nonetheless, the potential of repetitive DNAsequences was demonstrated in several studies. For instance, Staginnus et al. (1999) isolated the two tandemly organized chickpea-specific repeats from a genomic library (CaSat 1, CaSat 2) which were very informative. CaSat 1 defined a large cluster of sites in the subtelomeric region of both chromosomes A and B, while CaSat 2 proved to be present at each of the eight centromeres.

Progress in genomic analysis has led to a broader understanding of cytogentic variations and the correlation of chromosome number, length, morphology and in addition played a vital role in an accurate estimation of genome size. The meiotic chromosome behaviour has been characterized in both *Cicer* cultivars and hybrids. By virtue of in situ hybridization a little information about a small number of DNA sequences that have been chromosomally localized is available. However, a detailed cytogenetic map is still under study, and the literature regarding the long-range molecular chromosomal organization of the genome is scanty. Flow cytometry is nowadays used for the sorting of chickpea chromosome and also paves the way toward advanced genome exploration. The advancement in chickpea cytogenetics has been slow, as compared to other agriculturally important crops. The main knowledge gaps exist regarding chromosome structure in the cultivated form as well as wild forms within the genus *Cicer*. Progress in filling these gaps should be pursued by gaining more information about the chickpea genomic sequence, molecular cytogenetics and the use of flow cytometry to assign the nuclear genome into its component chromosomes. The likelihood is that in the near future, the community will be in a position to better make use of the full range of genetic diversity present in the genepool and thereby to sustain the breeding of improved chickpea cultivars.

3.1.4 Nutritional Value and Importance

Chickpea and other pulse crops are important foods in many nations and play a vital role in the diet of undernourished people around the globe. Pulses, in combination with cereals, are considered a nutritionally balanced human diet. Most health and nutrition organizations have recommended the regular consumption of pulses for better human health (Leterme 2002). Among the pulses, chickpea is considered a good source of energy, proteins, minerals, vitamins, fiber, and also contains potentially health-beneficial phytochemicals. Energy values for chickpea have been reported at 15–19 MJ/kg for kabuli types and 14–18 MJ/kg for desi types. The protein content of chickpea seed is 12.6–29.0% and 16.7–30.6% for kabuli and desi types, respectively. Krishna (1975) reported the use of chickpea seeds to treat protein deficiency diseases such as kwashiorkor. Chickpea seeds possess a balanced amino acid composition rich in lysine but contains less methionine and cysteine. Therefore, chickpea is considered an ideal companion to cereals, which contain a higher content of methionine and cysteine and less lysine. In addition to high protein, chickpea is also rich in lipids which mainly comprises of 62–67% polyunsaturated, 19–26% mono-unsaturated and 12–14% saturated fatty acids. The total lipid content of desi type range is 2.9–7.4% while in kabuli type the range is 3.4–8.8%. The major fatty acids in chickpea are linoleic and oleic, which are essential for normal growth and development. Carbohydrates are the main nutritional constituent in chickpea, with 51–65% in desi type and 54–71% in kabuli type. The monosaccharides which are predominant in chickpea include glucose, fructose, ribose and galactose while the abundant disaccharides include sucrose and maltose (Sanchez-Mata et al. 1998). The most important oligosaccharides in chickpea are raffinose, stachyose, ciceritol and verbascose. Ciceritol is the most abundant oligosaccharide, hence the name *cicer,* while starch is the main polysaccharide in chickpea. Chickpeas contain all the essential mineral such as Ca, Mg, K, P, S, Cl, B, Fe, Mn, Zn, Cu, Ni and Mo, which are vital for normal human growth and development (Grusak and Penna 1999). Chickpea is also a good source of vitamins such as A, B-complex, C, K and E. The nutritional composition of chickpea is given in Table 3.1.

Chickpea is consumed in the human diet as well as used for livestock feed. Chickpeas have a low glycemic index due to higher fiber and protein content and hence may help in reducing human body weight and the risk of fatal cardiac diseases. Chickpea seeds are used in raw and processed forms such as green vegetables, fried, roasted, broiled in snack foods and condiments (Parveen 2006). Chickpea also possess good medicinal value and plays a role in the prevention and treatment of many chronic diseases. In a nutshell, chickpea is a pulse crop of paramount importance and possesses huge nutritional and health benefits (Jukanti et al. 2012).

Table 3.1 Nutritional value of chickpea, raw (dry weight) per 100 g. Nutrient values and weights are for edible portion

Nutrient	Per 100 g	Nutrient	Per 100 g
Water	7.68	Leucine	1.465 g
Energy	378	Lysine	1.377 g
Protein	20.47	Methionine	0.270 g
Carbohydrate	62.95	Phenylalanine	1.103 g
Fiber, total dietary	12.2	Proline	0.849 g
Sugars, total	10.7	Serine	1.036 g
Calcium, Ca	57	Threonine	0.766 g
Iron, Fe	4.31	Tryptophan	0.200 g
Magnesium, Mg	79	Tyrosine	0.512 g
Phosphorus, P	252	Valine	0.865 g
Potassium, K	718	Fat	6.04 g
Sodium, Na	24	Saturated fatty acids	0.603 g
Iron, Fe	2.76	Butanoic acid	0.000 g
Phosphorus, P	4	Decanoic acid	0.000 g
Potassium, K	0.477	Dodecanoic acid	0.000 g
Sodium, Na	0.212	Hexadecanoic acid	0.508 g
Niacin	1.541	Hexanoic acid	0.000 g
Vitamin B-6	0.535	Octadecanoic acid	0.086 g
Folate, DFE	557	Octanoic acid	0.000 g
Vitamin B-12	0	Tetradecanoic acid	0.009 g
Vitamin A, RAE	3	Monounsaturated fatty acids	1.377 g
Vitamin A, IU	67	Docosenoic acid	0.000 g
Vitamin E (alpha-tocopherol)	0.82	Eicosenoic acid	0.000 g
Vitamin D (D2 + D3)	0	Hexadecenoic acid	0.012 g
Vitamin D	0	Octadecenoic acid	1.365 g
Vitamin K (phylloquinone)	9	Polyunsaturated fatty acids	2.731 g
Cholesterol	0	Docosahexaenoic n-3 acid	0.000 g
Amino Acids	7.68	Docosapentaenoic n-3 acid	0.000 g
Alanine	0.882 g	Eicosapentaenoic n-3 acid	0.000 g
Arginine	1.939 g	Eicosatetraenoic acid	0.000 g
Aspartic acid	2.422 g	Octadecadienoic acid	2.629 g
Histidine	0.566 g	Octadecatetraenoic acid	0.000 g
Isoleucine	0.882 g	Octadecatrienoic acid	0.102 g

Source: USDA, National Nutrient Database for Standard Reference 2018; https://www.nutrition-value.org

3.2 Cultivation, Production Constraints and Breeding Objectives

3.2.1 Adaptation and Cultivation

Chickpea is grown mostly in the semiarid regions of South Asia and its cultivation has increased to a great extent in Australia and Canada, to meet increasing export market demands from developing countries, including India and Pakistan. Even though it is suitable for production in low-fertility soils, mineral nutrient deficiencies often limit chickpea yield. Chickpea is known as a hardy crop, due to its ability to grow even in marginal soils. It is grown on a wide range of soils varying in texture from sandy to clay, generally with a low organic carbon content. Chickpea performs well when grown on sandy and loam soils with good drainage. In sandy loam soils, desi types require 30–45 kg N/ha, whereas kabuli types are usually nonresponsive (Walley et al. 2005). Post-flowering N fertilization is highly recommended in chickpeas exposed to drought stress which reduces the net photosynthetic rate and nutrient mobilization to reproductive parts. Phosphorus and potassium are also used and these are believed to increase the overall yield per plant. The amount of fertilizer required for optimal growth and development depends upon the soil type and climate conditions. Weeding should be done between planting and also during the growing season. Weeding can be carried out by two methods, either mechanical or chemical, or by traditional hand weeding in small fields. Chickpea can be grown successfully in soils varying in a pH range of 6–9. Acidic soils affect the availability of minerals and also cause severe toxicity. Kay (1979) reported an increased incidence of disease in chickpea growing in acidic soils. Alkaline soils reduce the growth of plant at seedling stage and also hamper the yield and overall productivity (Ahlawat et al. 2007).

Agro-climatic conditions and cropping systems in which chickpea is cultivated vary across growing regions. Chickpeas are grown mainly as a winter crop and require cool climate for growth and high temperature for maturity, but can also be grown in autumn and spring seasons in various regions in several different sequential and intercropping systems. In the Mediterranean area, a 2-year rotation of wheat/chickpea is more economical in both yield and soil organic matter than wheat-fallow. In South Asia, particularly in India, chickpea is commonly grown as an intercrop with cereals such as wheat, barley and mustard, as well as with oil crops like linseed and safflower. Variations in cropping system and crop season differ, region to region, and influence the crop productivity significantly. Harvesting is usually done when plants begin to turn yellow, and the lower pods turn brown to yellow brown in color.

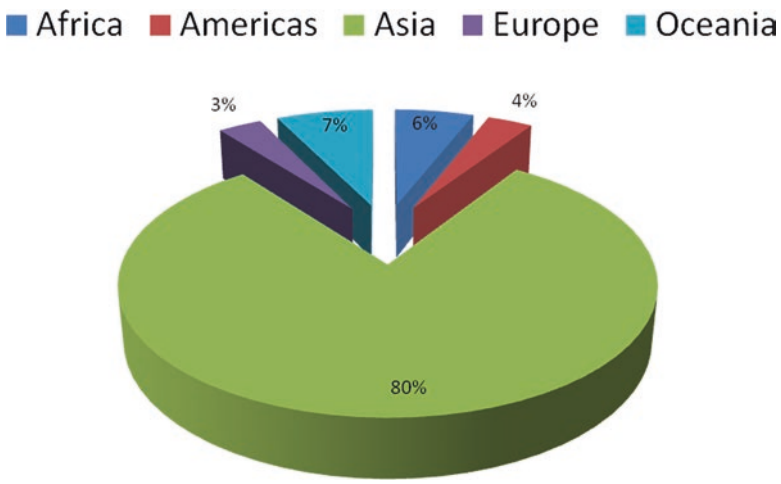

Fig. 3.2 Production share of chickpea by regions (2016). (Source: FAOSTAT 2018)

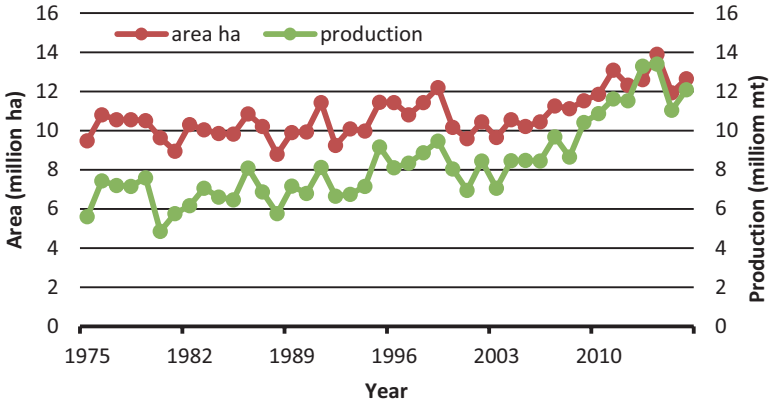

Fig. 3.3 Chickpea worldwide, area harvested and production, 1975–2016. (Source: FAOSTAT 2018)

3.2.2 Production Statistics

Globally, chickpea is mainly grown in developing countries, which account for ~97% of world area and 96% of world production. Figures 3.2 and 3.3 shows how much area is under cultivation, average yield and gross productivity of chickpea at the global level. In Africa, Ethiopia; in Europe, Spain; in North America, Mexico and in West Asia, Iran and Turkey have emerged as major chickpea-producing countries.

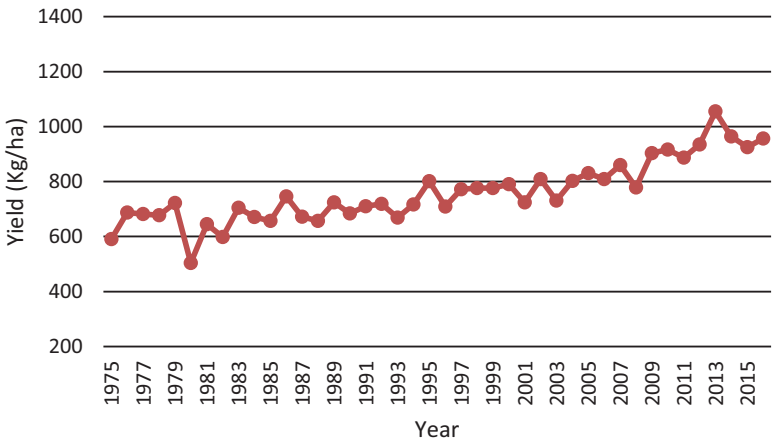

Fig. 3.4 Annual worldwide chickpea yields (kg/ha) and trend line, 1961–2013. (Source: FAOSTAT 2018)

Asia accounts for 87 % of the global area and 80% of the global production. Africa and North and Central America contribute 6, 2 and 1 % of the global production, each from 5, 2 and 1% of global area, respectively, while Oceania accounts for 5% of the area and 7% production (Fig. 3.2). South America contributes a mere 1% of global production harvested from 1% of the global area (FAOSTAT database 2016).

In 2016, the world chickpea production was 12.09 million mt on area of 12.65 million ha. During the last 40 years, global production of chickpea has increased from 5.2 million mt in 1975 to 12.09 million mt while the area under harvest increased from 9.5 million ha in 1975 to 12.65 million ha in 2016. In the last decade total production has increased to 13.4 million mt while the area under harvest has also expanded from nearly 12 million ha to 13.9 million ha in 2014 (Fig. 3.3).

The global yield of chickpea has steadily increased from 591 (1975) to 956 kg/ha (2016) with an increase of more than 6 kg/ha per annum as shown in Fig. 3.4. This increase in production is mainly due to the development of improved cultivars which are high yielding and widely adaptable to a broad range of agro-ecosystems. This crop is grown on all habitable continents. However, the bulk of production is centered in South Asian countries including India, Pakistan and Bangladesh.

India is the leading chickpea producing country with 70% of total world production. Fig. 3.5 shows India dominates the global chickpea production and the relative significance of the other major producing countries. Australia and Myanmar are the next most important producers, account for 8 and 5% of world production, respectively. The other major producing countries such as Pakistan, Turkey, Ethiopia and Iran account for 4% each. Other important producing countries include Argentina, Russia and Iran.

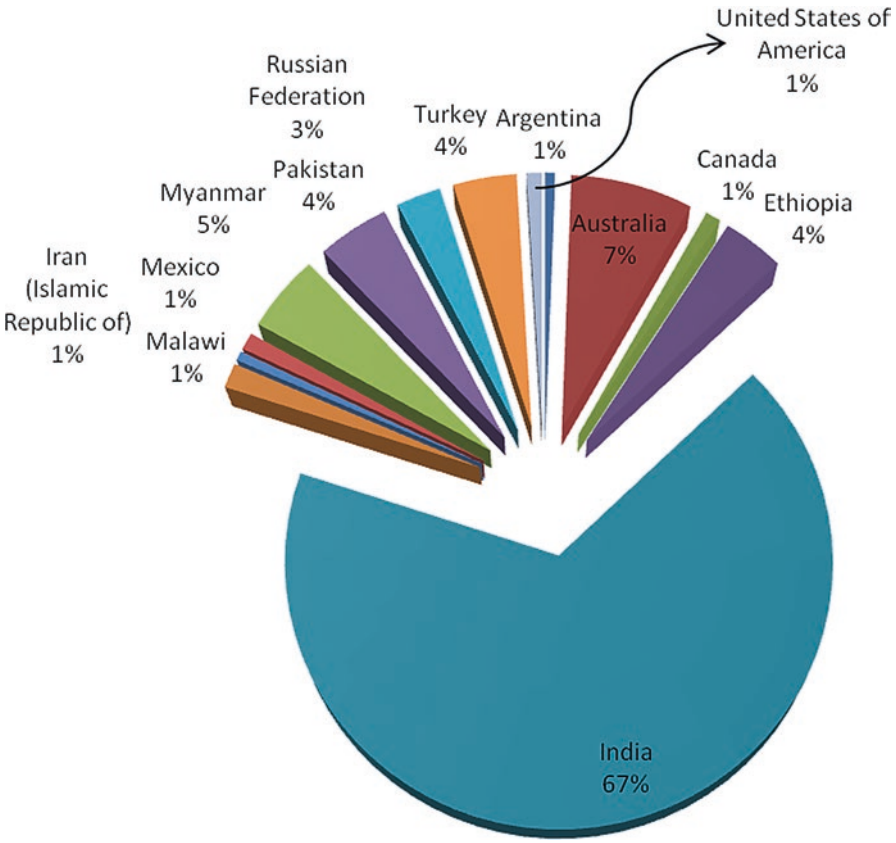

Fig. 3.5 Production share of chickpea by leading countries (2016). (Source: FAOSTAT 2018)

3.2.3 Production Constraints

At present the average global yield of chickpea is 0.9 mt/ha (FAO 2014), which is very low compared to its estimated potential of 6mt/ha under favorable growth conditions (Singh 1985). The main constraints that limit desired goals of chickpea productivity include low genetic variability, low and unstable yield and low resistance to biotic and abiotic stresses. These biotic and abiotic factors play a critical role in reducing the inherent potential for genetic improvement of yield traits of many pulse crops including chickpea (Yankova and Sovkova-Bobcheva 2009). Diverse biotic stresses in combination with other local factors are hampering global yield of chickpea, particularly in resource-poor areas (Singh et al. 2014a, b, c). Wilt is caused by the soil fungus *Fusarium oxysporum* incurs yield loss of 10–90 % (Jimenez-Diaz et al. 1989; Singh and Reddy 1991). *Ascochyta* blight, a foliar disease of chickpea, caused by *Ascochyta rabiei*, affects chickpea production by reducing the production 10–100 % (Nene and Reddy 1987; Singh 1990). This disease occurs in chickpea growing countries; India is the most affected. Other biotic

stresses such as pod borer, aphids, cutworms, powdery mildew, rust and wilt are the major pest and diseases affecting chickpea production.

Legumes possess the ability to fix nitrogen biologically and hence are rich in nitrogen and phosphorus that attract a wide range of insect pests and insect-borne diseases (Sinclair and Vadez 2012). Among the various insects drastically reducing production, the pod borer *Helicoverpa armigera* is the most damaging. It is fairly prevalent in important chickpea growing regions of Asia, Africa and Australia. Bruchids such as *Callosobruchus chinensis* also affects stored chickpeas in India and is responsible for 13% reduction in production. *Botrytis* gray mold caused by *Botrytis cinerea* Pers. ex Fr. (Teleomorph: *Botryotinia fuckeliana* Grover and Loveland) is a shattering disease of chickpea in South Asian countries including India, Pakistan, Bangladesh and Nepal, and significantly damages crops in Argentina, Australia, Canada, Myanmar, Mexico, Columbia, Hungary, Spain, Vietnam and the USA. The disease frequently causes total yield loss in India, Nepal and Bangladesh (Pande et al. 2005; Singh and Sharma 2002). Argentina has witnessed loss of about 96% (Carranza 1965). Dry root rot caused by *Macrophomina phaesolina* (Maub.) Ashby is a serious problem in the chickpea growing regions of Australia, India, Ethiopia, Iran, Pakistan, Bangladesh and Nepal (Nene et al. 1991; Singh and Sharma 2002). *Sclerotinia* stem rot (or white mold) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is prevalent in almost all the chickpea producing areas. It is widely prevalent in Australia, Bangladesh, Chile, India, Iran, Morocco, Nepal, Pakistan, Syria, the USA and Tunisia. Abiotic limiting factors include drought, temperature stress like cold and high temperatures that hamper chickpea production. The primary abiotic stress is cold, which occurs when the mean day temperature falls below 15 °C and it increases the flower drop and pod abortion, which consequently results in major production loss (Nayyar et al. 2005; Singh et al. 2014a, b, c; Srinivasan et al. 1999). The second most important abiotic stress is drought, particularly severe drought which incurs huge losses in global chickpea production (Devasirvatham and Tan 2018; Khanna-Chopra and Sinha 1987). The other abiotic stress is heat that occurs in combination with or overlapping drought stress (Toker and Canci 2009). In South Asian countries chickpea is exposed to drought along with high temperature as it is grown in the post-rainy season. Basu et al. (2009) are of the opinion that by the end of 2050 the temperature is predicted to rise by 3–4 °C. Albeit a winter season crop in northern India, chickpea faces heat stress (>35 °C) during the flowering phase and hence the most affected plant parts include flowers, pods and seeds (Toker and Canci 2006; Wery et al. 1993). Heat stress affects pod formation and hence seed set and results in huge production loss (Basu et al. 2009; Kumar et al. 2013a, b; Summerfield et al. 1984; Wang et al. 2006).

3.2.4 Breeding Objectives

Chickpea breeding objectives generally differ depending on the problems and priorities of farmers, and consumer preferences of the specific region. The productivity of chickpea is usually low, with the average yield ranging from 908 kg/ha in Asia,

934 in Europe, 1174 in Africa, in North America and 1833 kg/ha in Central America (FAO 2016). These figures indicate the need for genetic enhancement of yield and to achieve desired production goals. Muehlbauer et al. (1993) reported that the main breeding goals are higher and more stable seed yield, extended adaptability, biotic and abiotic stress resistance and better seed quality. The major concern for any chickpea breeding program is its narrow genetic base which imposes a lesser degree of genetic variability and potential genetic gain. Therefore, to make breeding efforts more effective, a broadening of the genetic base of chickpea is very much required. Both gene introgression from wild species and induced mutagenesis can be used to enhance the genetic variability in cultivated chickpea. Wild chickpea is considered an important repository of genes that impart resistance to a diverse range of biotic and abiotic stresses. Cultivated chickpea is a source of genes that can impart resistance to several biotic (*Fusarium* wilt, *Ascochyta* blight) and abiotic (drought, heat) stresses (Gaur et al. 2010). Several mutant cultivars have been used directly, and others indirectly, as parents in crossbreeding programs (Gaur et al. 2007). Singh et al. (2014a, b, c) reported few useful agro-morphological traits in wild chickpea and advocated usefulness through introgressions to widen the genetic base of cultivated chickpea. Mutants with improved traits, such as increased number of flowers per node (cymose inflorescence) (Gaur and Gour 2002), brachytic growth habit (Gaur et al. 2008) and determinate growth habit (Hegde 2011), have been induced in chickpea and act as genetic resource to be used in the creation of elite chickpea cultivars.

3.3 Germplasm Diversity and Conservation

3.3.1 Germplasm Diversity

The worldwide chickpea collection comprises 99,877 accessions preserved in 120 genebanks spread across 64 nations. These accessions also comprise 1476 wild *Cicer* types. The list of major genebanks holding chickpea collections greater than 1000 accessions is presented in Table 3.2. In total they possess 87,341 accessions; 98.3% cultivated and 1.7% of wild *Cicer* types. *Cicer arietinum* is the only cultivated species among the 43 species in the genus *Cicer*. Globally the species comprises thousands of landraces and a huge number of cultivars grown in over 50 countries. In recent years the preferred cultivation of genetically-modified over traditional cultivars has led to a reduction in diversity within the species. Additionally, anthropogenic activities such as increased deforestation, expanding urbanization, cultivation of exotic plant species and natural calamities have also contributed to the reduction in species variability. These factors necessitate the availability of characterized germplasm for breeding superior cultivars.

Table 3.2 Genebanks holding more than 1000 chickpea germplasm accessions

Institute	Wild *Cicer*		Cultivated	Total
	Species	Accession		
Australian Temperate Field Crops Collection (ATFCC), Horsham Victoria, Australia	18	26	8409	8435
Institute of Biodiversity Conservation (IBC), Addis Ababa, Ethiopia	–	–	1173	1173
Institute for Agrobotany (RCA), Tápiószele, Hungary	5	9	1161	1170
International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India	19	308	20456	20764
National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India	10	69	14635	14704
National Plant Gene Bank of Iran, Seed and Plant Improvement Institute (NPGBI-SPII), Karaj, Iran	-	-	5700	5700
Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas (INIA-Iguala), Iguala, Mexico	–	–	1600	1600
Plant Genetic Resources Program (PGRP), Islamabad, Pakistan	3	89	2057	2146
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR), St. Petersburg, Russia	–	–	2767	2767
International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria	11	540	15194	15734
Plant Genetic Resources Department, Aegean Agricultural Research Institute (AARI), Izmir, Turkey	4	21	2054	2075
Institute of Plant Production n.a. V.Y. Yurjev of UAAS, Kharkiv, Ukraine	–	–	1760	1760
Western Regional Plant Introduction Station, USDA-ARS, Pullman, WA USA	21	194	7844	8038
Uzbek Research Institute of Plant Industry (UzRIPI), Botanica, Uzbekistan	–	–	1055	1055
Total	N/A	1256	85865	87121

Source: http://www.fao.org/wiews-archive/germplasm_query.htm?i_l=EN

The huge number of landraces, cultivars and varieties represent a wealth of alleles and can be incorporated into the breeding programs with the objectives to improve yield stress resistance and adaptability (Fig. 3.6).

The ICRISAT genebank maintains the largest number of chickpea accessions with more than 20,000, collected from 60 nations, including 308 wild accessions. Of the 308 wild chickpea accessions, 6 countries provided 233 accessions; 75 from Afghanistan, Turkey, Syria and Pakistan. The ICRISTAT genebank maintains 4153 accessions obtained from 65 collecting missions in 15 nations and the remaining were donations from 19 countries.

The Indian National Genebank, New Delhi, preserves 14,651 accessions of chickpea. A core set of 1103 accessions has been maintained by employing allelic richness, 70.0% of which were of Indian origin (Archak et al. 2016). Shannon-Weaver diversity indices indicate that the chickpea core harbors greater diversity as

Fig. 3.6 Genetic diversity in seeds of chickpea. (Source: Photo by ICRISAT https://cropgenebank.sgrp.cgiar.org/index.php/crops-mainmenu-367/chickpea-mainmenu-360/characterization-mainmenu-373)

compared to the whole collection in agro-morphological traits, which inturn reflects that the chickpea core maximized the phenotypic diversity available in the Indian chickpea germplasm.

3.3.2 Germplasm Conservation Appraoches

To meet an adequate food demand of a rapidly growing population the conservation of germplasm and existing variability in germplasm lines is of great significance.

3.3.2.1 Cold Storage

At ICRISAT conservation of chickpea base germplasm is maintained for long-term storage at −20 °C; however, the 4 °C and 30% RH is employed for the active collection which is maintained for medium-term storage. Seed moisture content is crucial and varies according to the collection. For active collection about 10% moisture content is maintained during seed processing for storage, while 6–7% moisture content is maintained for base collections. Special aluminum screw cap containers are used for the active collection. About 350 g of chickpea seed per accession is preserved as the active collection. Vacuum-sealed standard aluminum foil pouches are used for conserving seeds in the base collection (Upadhyaya and Gowda 2009).

3.3.2.2 Seed Regeneration

Seed regeneration is an imperative feature of managing genetic resources. Chickpea, being self-pollinated, can easily be regenerated. Regeneration is employed when seeds in an active collection are below 75 g or when the seed viability is less than 85%; in the base collection the accessions are regenerated when seed viability is less than 90%. About 100 plants are grown in rows to collect and preserve seeds for regeneration. Proper monitoring is employed during the cropping season to distinguish and remove off-type plants. Upadhyaya and Gowda (2009) recorded the data agro-morphological traits (growth habit, flower size, seed color and seed shape) of chickpea during regeneration and compared it to early-generated passport information to ensure the reliability of each accession.

3.4 Conventional Breeding Methodologies

Chickpea is a predominantly self-pollinated crop and harbors little genetic variability. The creation of genetic variability, effective selection and subsequent evaluation of selected lines are the three basic steps of a breeding program. Genetic variability forms the basis for any crop improvement program and determines the success of a breeding program. The conventional breeding approach is employed for the creation of genetic variability, including plant introduction and hybridization.

3.4.1 Plant Introduction

Plant introduction is a method of identifying, obtaining and evaluating genotypes from within and/or outside the country and screening them for desirable genotype with higher yield and better adaptability to the local environment. The efficiency of plant introduction depends on the nature of the introduced material and its original habitat. Generally, plant introduction is often found to be more effective if the material is introduced from regions with comparable agro-climatic conditions. The following ways are employed for plant introduction:

(a) Exchange of material with fellow plant breeders;
(b) Exploration of areas showing species richness and diversity;
(c) Obtaining generic resources from international organizations like FAO, ICRISAT, ICARDA and USDA plant introduction stations.

Introduced material may be either homozygous or heterozygous segregating populations. There is no problem of adaptability in the case of homozygous lines; however, in the case of a heterozygous population it is necessary to screen and isolate lines with traits of interest that will be useful in a recombination breeding program. Among all breeding approaches, plant introduction is considered to be a

cheap and quick way of developing new cultivars. There are several examples of successful introduction in chickpea; one noteworthy example is a bruchid resistant-line (G109-1) introduced to India from Turkey (Saxena and Raina 1970).

3.4.2 Hybridization

Hybridization is carried out to combine desirable traits from two or more parents, particularly from wild relatives, into a single cultivar. The wild relatives of the crop are considered critical resources for the improvement of many traits, particularly seed yield, in various crops (Stalker 1980). In chickpea yield genes have been introgressed from *Cicer reticulatum*, *C. echinospermum* (Singh et al. 2005; Singh and Ocampo 1997) and *C. pinnatifidum* (Sandhu et al. 2006; Singh et al. 2012a, b, c). Successful interspecific crosses between *C. arietinum* and *C. reticulatum* was achieved and later hybridization was also carried out between *C. arietinum* and *C. echinospermum*, by several researchers (Ladizinsky and Adler 1976; Pundir and Mengesha 1995; Singh and Ocampo 1993).

3.4.2.1 Selection of Parents

The appropriate selection of parents is important as it largely determines the success or failure of hybridization programs. The choice of parents varies depending upon the main objectives to be accomplished; if the objective is to create a superior cultivar, then cultivars with better adaptability to local environment are chosen as one parent and the second parent is chosen only to complement the first parent. However, if the objective is to enhance the degree of genetic variability, then diverse cultivars are selected as parents. For effective and efficient selection of the parents, biometrical approaches are employed to analyze combining ability of genotypes and the diversity. The preliminary knowledge on the selection of parents for a crossing program based on specific objectives can be obtained from the national gene banks, e.g. National Bureau of Plant Genetic Resources (NBPGR), international institutes and other national organizations.

3.4.2.2 Crossing Techniques

Crossing in chickpea is a tedious job with a low success range of 10–50%, depending upon the genetic constitution of the crossing parents and the environmental conditions such as temperature and humidity. Several researchers like, Van der Maesen (1972), Smartt (1976) and Auckland and van der Maesen (1980) have reviewed artificial hybridization techniques in chickpea. Emasculation and pollination is also difficult to carry out due to the extremely small size of flowers that results in damage to other floral parts and reduction in the success rate. Apart from

this, the success of artificial hybridization also depends upon the timing of pollination and fertilization. Emasculation in the afternoon followed by pollination the following morning is preferred in low temperature conditions, while under the conditions of high temperature morning emasculation followed by immediate pollination gives better results (Bejiga and Tessema 1981; Khosh-Khui and Niknejad 1972; Pundir and Reddy 1998; Salimath et al. 2007; Singh and Auckland 1975). It has been documented that pollen grains take 30 min for germination, 4 h to reach the base of ovary (Malti and Shivanna 1983) and another 24 h for fertilization after pollination. The precautionary measures to be taken during hybridization are as follows:

(a) Large flower buds are preferred;
(b) Lateral buds are selected (Sindhu et al. 1981);
(c) Emasculation and pollination free from mechanical injury to flowers;
(d) Hybridization just after the formation of the first pod (Bahl and Gowda 1975).

In 1930 the first recombination breeding was carried out in chickpea with the main aim of transferring disease-resistance traits from a F8 into the Pb7 generation and the results were outstanding, with the development of the first *Ascochyta* blight-resistant cv. C12/34. In recombination breeding, selections are made as per pedigree/bulk methods or their modifications. On the basis of main objectives, hybridization involves the following crosses.

Single Cross In this type of cross only two types of parents are involved in the development of cultivars with improved traits. The traits improved were mainly resistance against biotic and abiotic stresses. The cultivars developed through a single cross were resistant against wilt and blight, high yielding and were widely adapted to a range of agro-ecosystems in India.

Three-Way Cross In this type of cross three parents, each with a specific desirable trait are used with the aim of recombining the traits together in a new cultivar. A cross is made between the two parents to raise the F1 generation and then a back-cross is carried out between F1 and the other parent. A three way cross is more beneficial as compared to a single cross as it provides additional opportunity for gene interaction and leads to creation of cultivars with more genetic variability. Three-way crosses can be managed by pedigree or bulk-breeding methods. Thus, it takes more time to isolate uniform progenies. A number of elite cultivars with improved traits were developed in chickpea through three-way crosses. In 1985 cv. BG244, showed resistance to blight and stunt disease and cold stress was developed by three crossing involving parents ([850-3/27 × P922] × P9847 kabuli type. In 1999 another high yielding cv. BGD72 was released by a three-way cross involving parents ([BG256 × E100Y] × BG256). Another cv. C214, was developed from cross ([G24 × IP58] × G24) with drought-resistance and cold tolerance. Another cv. JKG-1, was released in 2002 from a cross ([ICCV2 × Surutato] × ICC7344) and exhibited large-seeded kabuli type seeds (Dua et al. 2001; Singh 1987).

Multiple Crosses In this type of cross more than three parents are employed for the improvement of a single trait of an otherwise outstanding cultivar. The main aim behind a multiple cross is to enhance the degree of genetic variability. Resultant populations are managed by the pedigree or bulk-breeding methods. Cultivars developed from multiple crosses show better adaptability to a range of agro-ecosystems. In 1984 cv. BG256, was developed from a cross involving four parents ([JG62 × 850-3/27] ×[L550 × H208]) which showed improvement in wilt and blight-tolerance, and seed size. In 2002, cv. Phule G 95311, was developed from a cross involving parents ([ICCC32 × ICCL 80004] ×[ICCC49 × FLIP 82K] × ICCV3) and reflected improvement in seed size. In 1987 a double podded and wilt tolerant cv. SG2, was developed from a cross involving parents ([E100Y × P436] × [L550 × F378]) (Dua et al. 2001; Singh 1987)

3.4.2.3 Handling of Segregating Populations

Diversity and the combining ability of parents are the two important aspects that need to be taken care of while performing a cross. The handling of the segregating populations is important for achieving better results as it is well correlated with the heterosis of F1 hybrids. Only a few but promising hybrids showing high heterosis and less in-breeding depression are advanced for future generations to maintain sufficiently large F2 populations, while rejecting the poor performing F1 crosses. The most widely employed method of handling segregating population in chickpea is the pedigree method of selection (Lal et al. 1973) whereas Byth et al. (1980) reported that this method is less suitable, and recommended the bulk method; the single-seed descent method has been advocated by Singh and Auckland (1975).

While taking into consideration the merits and demerits of different methods of handling segregating populations in chickpea, Rahman and Bahl (1985) concluded that mass selection is more suitable as compared to bulk and single-seed descent methods. Singh and Waldia (1994) are of the opinion that the development of high-yielding and short-duration cultivars is best done by the bulk method of breeding. However, the development of cultivars with improved resistance to biotic stresses is more suitable in the pedigree method; drought tolerance and winter hardiness in the bulk-pedigree method; abiotic stresses, seed size, earliness and plant type is suitable for the modified bulk method (Singh 1987).

3.5 Mutation Breeding

Historically, an ancient book entitled *Lulan* mentions the existence of spontaneous cereal mutants in China around 300 BC (Van Harten 1998). The concept of using induced mutations in plant breeding programs to create novel cultivars was first reported by De Vries (1901). About three decades later, the practical significance of mutation breeding was demonstrated by Stadler (1928) in barley and Goodspeed

(1929) in *Datura* and *Nicotiana*. A definite authentication that electromagnetic waves could induce mutations in *Drosophila* and maize was advocated by Muller (1927) and Stadler (1928), respectively and they concluded that it was possible to enhance the rate of spontaneous mutations through irradiations. Similar results have been reported by Ganger and Blakeslee (1927) in *Datura stromonium.* The first example was a mutant of *Nicotiana tabacum* called Chlorina that was generated through the treatment of floral buds with X-rays beginning in the 1930s (Coolhaas 1952; Konzak 1957; Tollenaar 1934, 1938). After pioneering reports on the use of mutation breeding in crop improvement, induced mutations were exploited by many scientists worldwide. However, the applicability of mutation breeding gradually waned towards the 1960s due to many of its negative effects (Allard 1960). Thereafter, with the development of international coordination and availability of financial assistance from FAO/IAEA to research organizations, systematic work began on mutation breeding using various crops, including chickpea.

3.5.1 Mutagens and Their Mode of Action

Comprehensive accounts by Sharma (1985), Van Harten (1998), Micke (1995) and Kodym and Afza (2003) on the basic mutational process and mode of action of mutagens, contributed to the present knowledge on the subject. Many researchers have also reviewed the properties and action of physical and chemical mutagens in various crops (Al-Qurainy and Khan 2009; Amin et al. 2016; Gottschalk 1978a, b; Goyal and Khan 2010b; Kalapchieva and Tomlekova 2016; Nakagawa et al. 2011; Raina et al. 2018a, b) that considerably exaggerated the efficacy of induced mutations in crop improvement. Currently, mutagenesis has received great attention for use in a promising new technique known as TILLING (targeting induced local lesions in genomes). Mutagens are mainly of two types: (a) physical radiation and (b) chemical mutagens.

3.5.1.1 Physical Mutagens

Physical mutagens include gamma rays, X-rays, α-rays, β-rays, UV rays and neutrons. Gamma rays are the most favored physical mutagen by mutation breeders and are extensively used in crop improvement programs (Celik and Atak 2017). Gamma rays absorption and their impact on biological material are greatly influenced by species, cultivar, plant age, physiology and morphology of the plants, besides their genetic organizations and degree of irradiation (Çelik and Atak 2017; Laskar et al. 2018a). Stimulatory, moderate and damaging effects on plant growth and development are depending on employed dose and duration of mutagens and the targeted crop. The type of DNA modifications induced by physical mutagens includes base substitutions, base alterations, base deletions and chromosomal abnormalities. During the process of irradiation treatment of biological matter, these high energy

rays collide with atoms and emit electrons leaving positively charged ions or free radicals which may lead to wide genetic alterations (Van Harten 1998). These alterations modify almost all vital structural and functional biomolecules such as lipids and proteins; these alterations eventually affect diverse morphological, anatomical, biochemical, developmental and physiological processes of crop species (Kebeish et al. 2015). Physical mutagens have been the most successful mutagenic agent in inducing a broad spectrum of mutations in mustard (Javed et al. 2000); chrysanthemum (Momin et al. 2012); black cumin (Amin et al. 2019); chickpea (Kozgar 2012; Laskar et al. 2015; Raina et al. 2017); cowpea (Abu et al. 2006; Badr et al. 2014, Raina et al. 2018a, b; Thimmaiah et al. 1998); faba bean (Khursheed et al. 2017, 2018a, b), mungbean (Wani et al. 2017); fenugreek (Hassan et al. 2018) and lentil (Laskar et al. 2018b). In addition to morphological mutations, physical mutagens also induce a wide range of chromosomal aberrations in various crops such as faba bean (Khursheed et al. 2015, 2018c) and blackcumin (Amin et al. 2016). Reports on the combination treatments of gamma rays and sodium azide (SA) treatments to induce mutations are very scanty. However, Khursheed et al. 2016 reported an improvement of yield and mineral content in two cultivars of *Vicia faba* L. through combination treatments of gamma rays and SA.

3.5.1.2 Chemical Mutagens

Subsequent to the use of physical mutagens, the discovery of chemicals with mutagenic potential represented a milestone in the history of mutation breeding. A wide range of chemical mutagens employed, singly or in combination, successively or simultaneously with physical mutagens, are now known to induce mutations in various crop plants (Ahloowalia and Maluszynski 2001; Encheva 2009; Goyal and Khan 2010a; Khan et al. 2011; Konzak et al. 1965; Kozgar 2012; Laskar et al. 2018a; Saleem et al. 2005). Chemical mutagens have advantages over ionizing rays, owing to their relatively low cost, easy handling, milder effect and greater specificity (Auerbach 1965; Handro 1981; Salnikova 1995; Tantray et al. 2017; Wani et al. 2014). The conceptual knowledge on the fundamental aspects of mutational processes and the possible mechanism of action of several mutagens has widened significantly with the reports and reviews of Gottschalk and Wolff (1983) and Kodym and Afza (2003). Chemical mutagens have become the preferred method of induction of mutation, even with the dawn of modern technologies.

3.5.2 Basic Components of Mutation Breeding

The basic components of mutation breeding are (a) mutation induction, (b) mutation detection, (c) mutant testing and (d) official cultivar release. Mutation induction is carried out by exposing biological material to mutagens; it is quick, taking minutes or a few hours while mutation detection takes a few months, or even years, but

high-throughput screens are very useful to detect a mutation in less time. Mutant cultivar release takes about 10 years on average but can be accelerated using marker-assisted selection and other emerging biotechnologies (Joung and Sander 2013; Zheng et al. 2013). Gaul (1964) classified mutations phenotypically into two groups:

(a) Macromutations: A mutation that is phenotypically visible and morphologically distinct and produces a phenotype well outside the range of variation previously existing in the population.
(b) Micromutations: A mutation with a small effect which can be detected only by the aid of statistical analysis such as character mean, variance, heritability, etc. A majority of such mutations are in polygenically controlled traits; they are of greatest value to plant breeders because most economically-useful traits are polygenically controlled.

3.5.3 Induced Mutation Applications in Crop Improvement

Mutation breeding is considered as an efficient tool to create variability in a crop species in a very short span of time as compared to hybridizations. Brock(1977) reported that the average time required from the beginning of mutation treatment to the release of a mutant cultivars is approximately9 years; twice the time, 18 years, are required for a cultivar created from crossing programs. Additionally mutations are induced in both qualitative and quantitative traits, altering new alleles of known and previously unknown genes, and modify linkages (Konzak et al. 1977). Gustafsson (1947) was among the pioneers to report the utility of mutations for the genetic improvement of various crop plants. Several researchers supplemented comprehensive knowledge on the function and applicability of induced mutations for the improvement of genetic resources in several crop plants worldwide (Brock 1965; Chhun et al. 2003; Goyal and Khan 2010a; Ilbas et al. 2005; Kharkwal 1996; Kozgar et al. 2014; Nakagawa et al. 2011; Oladosu et al. 2015; Raina et al. 2017; Rajput et al. 2001; Toker 2009). Micke (1995) reported that the continued efforts of numerous workers over the years ultimately converted the randomness of induced mutation into a targeted event for specific economic benefits. Induced mutations have led to the development of cultivars with high genetic variability, good quality and high yield, thus enhancing agronomic inputs and wide farmer acceptance (Ahloowalia et al. 2004; Javed et al. 2016). In addition to the crop improvement, induced mutations are efficient tools to study the nature and function of the genes which regulate diverse developmental processes (Adamu and Aliyu 2007). The great advantage of mutation breeding is the scope of improving only one or two traits without affecting the entire genetic constitution of the crop plant (Shu et al. 2012). Many economically-important cultivars have been developed and released through mutation breeding (Ahloowalia et al. 2004; Raina et al. 2016). In genetic improvement of crops, induced mutations have shown promising results and offer a great potential to serve as a complementary approach (Mahandjiev et al. 2001).

3.5.4 Landmark Achievements in Mutation Breeding

Widespread use of induced mutations in crop improvement programs has led to the official release of 3275 mutant cultivars of 170 different plant species worldwide. Globally, so far 432 legume cultivars have been released and are in commercial cultivation and about 55 mutant legume cultivars have been developed in India up to 2017. Several countries, including India, have adopted mutation breeding as a tool for crop improvement. Of the total mutant cultivars, 27 mutant cultivars of chickpea have been developed and officially released. Toker and Cagirgan (2004) reported that chickpea, grown on marginal lands faces several abiotic and biotic stresses such as heat, salinity, cold, drought, insects and diseases that hamper its productivity in general. Hence it is very necessary to create cultivars that harbor a tolerance to a wide range of environmentally-induced stresses. Mutation breeding is considered an effective tool to develop and release chickpea cultivars that can withstand a wide range of stresses.

To achieve the desired goal of increased production, genetic variability also should be enhanced in the development of resistant cultivars. Mutation breeding has been widely employed for the enhancement in the degree of genetic variability. The details of some improved varieties of chickpea developed through induced mutations are given in Table 3.3. Gaur and Gour (2002) reported a flower mutant with nine flowers per node as compared to two flowers per node in the control population. Haq and Singh 1994, reported cv. M16119 which is both cold-tolerant and resistant to *Ascochyta* blight. Another cv. M 699 (Hyprosola) was developed in Bangladesh which was found to be early maturing. In India, four high-yielding, blight and wilt resistant chickpea mutant cvs. Pusa 408(Ajay), Pusa 413 (Atul), Pusa 417 (Girnar) and Pusa 547, were developed and officially released. Cultivar Pusa 547is high yielding with bold seeds and high tolerance to wilt and root rot complex (Kharkwal and Shu 2009). Some chickpea mutants (CM 88, CM 98) have resulted in large revenue gains in Pakistan (Ahloowalia et al. 2004).

The use of conventional breeding methods over a long period of time may have resulted in reduced cowpea genetic variability at present. Hence, enhancement of genetic variability is a prerequisite for any further improvement in a crop species. Mutation breeding is considered an effective tool to increase the genetic variability and hence mutations may be successful in plant-breeding programs (Micke 1988a, b; Raina et al. 2016).

3.6 Modern Breeding Methods

Traditional approaches of chickpea breeding have enhanced yield but did not achieve the desired goals of production. Conventional breeding approaches tend to assemble all the desired genes into a cultivar by carrying out phenotypic selection of traits. However, phenotypic selection of traits is cumbersome, involves

Table 3.3 Details of chickpea varieties developed through mutation breeding

Mutant cultivar	Country	Year	Short description
Hyprosola	Bangladesh	1981	Developed by irradiation with gamma rays (200 Gy). Main improved attributes of mutant cultivar are early maturity (10 days earlier), more pods, higher harvest index, higher planting density, high yield (19%)
Line 3	Egypt	1992	Developed by combined treatment with gamma rays (50 Gy) and EMS (0.025%). Main improved attribute of mutant cultivar is high yield
Kiran	India	1984	Developed by treatment with neutrons. Main improved attributes of mutant cultivar are erect plant type, increased pod number, high yield, early maturity and salt tolerance
Pusa 408 (Ajay)	India	1985	Developed by irradiation of seeds with gamma rays (600 Gy). Main improved attributes of mutant cultivar are high yield, blight resistance, semi-erect, 140–155 days to maturity and plant architecture
Pusa 413 (Atul)	India	1985	Developed by irradiation of seeds with gamma rays (600 Gy). Main improved attributes of mutant cultivar are high yield, wilt resistance, 130–140 days to maturity
Pusa 417 (Girnar)	India	1985	Developed by irradiation of seeds with gamma rays (600 Gy). Main improved attributes of mutant cultivar are high yield, short, semi-erect, profusely branched, high pod number, 110–130 days to maturity, wilt resistance
CM 72	India	1985	Developed by irradiation with gamma rays (150 Gy). Main improved attributes of mutant cultivar are resistance to (*Ascochyta rabiei*) and high yield
NIFA-88 (CM-1918)	Pakistan	1983	Developed by irradiation of seeds with gamma rays (100 Gy). Main improved attribute of mutant cultivar are moderate resistance to *Ascochytarabiei*, 2 weeks earlier maturity, high yield (15–20%), higher nitrogen amount fixation
CM-88	Pakistan	1990	Main improved attributes of mutant cultivar are resistance to *Ascochyta*blight and*Fusarium*wilt, and high yield
CM-98	Pakistan	1994	Developed by irradiation with gamma rays (300 Gy). Main improved attributes of mutant cultivar are resistance to *Ascochyta* blight and wilt
NIFA-95	Pakistan	1998	Developed by irradiation with gamma rays (200 Gy). Main improved attribute of mutant cultivar is resistance to bacterial blight
Hassan-2K	Pakistan	1995	Developed by irradiation of seeds of kabuli type exotic chickpea cv. ILC-195 with gamma rays (450 Gy). Main improved attributes of cultivar are high yield, higher protein content (24%) and resistance to blight and wilt
CM 2000	Pakistan	2000	Developed by irradiation of with gamma rays (150 Gy). Main improved attributes of mutant cultivar are high yield and resistance to diseases

(continued)

Table 3.3 (continued)

Mutant cultivar	Country	Year	Short description
TAEK-SAGEL	Pakistan	2000	Developed by irradiation with gamma rays (150 Gy). The mutant chickpea cv. TAEK-SAGEL had been tested in 2004–2005 and the main improved attributes are early maturity (95–100 days), higher yield capacity (180– 220 kg/ha), *Ascochyta* blight resistance
THAL-2006	Turkey	2006	Developed by hybridization with mutant line. Main improved attributes of mutant cultivar are tolerance to blight, tolerance to moisture stress and bold seed size
CM-2008	Pakistan	2006	The mutant cv. CM-2008 was officially approved in 2008. It was developed by treatment with chemical mutagen 0.2% EMS. Main improved attributes of mutant cultivar are seed size, resistance to wilt and high yield
Binasola-3	Pakistan	2008	Developed by 200 Gy gamma irradiation of dry seeds of exotic genotype G-97 to create variability and followed by selections in later generation. Main improved traits are early maturity, erect plant type, larger seed size and rough seed coat
Binasola-4	Bangladesh	2001	Developed by hybridization with one ICRISAT line K-850 and mutant cv. Hyprosola obtained by irradiation of seeds with gamma rays (200 Gy), made selections from F2 and onward generations. Main improved attributes are higher seed yield, medium seed size and bright seed coat color
Pusa 547	Bangladesh	2001	Developed by irradiation with gamma rays (600 Gy). Main improved attributes of mutant cultivar are high yield, good cooking quality, tolerance to *Fusarium* wilt, stunt virus and root rot
BGM 547	India	2006	Developed by irradiation with gamma rays. Main improved attributes of mutant cultivar are high yield and moderate resistance to *Helicoverpa armigera*.
Binasola-2	India	2005	Not available
Binasola-5	Bangladesh	1998	Seeds of Hyprosola (mutant cv.) were treated with gamma-rays with doses of 150, 200, 250, 300, 350 and 400 Gy. This mutant performed better than the controlcv. BARI Sola-3. The mutant was registered as cv. Binasola-5 for commercial cultivation in Barind (dry prone) areas in Bangladesh
Binasola-6	Bangladesh		Mutant cv. Binasola-6 was developed by hybridization with one ICRISAT line K-850 and one advanced mutant G-299 obtained by irradiation of seeds with gamma rays (200 Gy), made selections from F2 and onward generations. Main improved attributes are higher seed yield, medium seed size and attractive straw seed coat color. Considering all these, the mutant was registered as cv. Binasola-6 for commercial cultivation in Barind (dry prone) areas in Bangladesh

(continued)

Table 3.3 (continued)

Mutant cultivar	Country	Year	Short description
Binasola-7	Bangladesh		Mutant cv. Binasola-7 was developed by irradiation of seeds of Binasola-2 with gamma rays (200 Gy), made selections from M2 and onward generations. Main improved attributes are higher seed yield, medium seed size, deep green leaves and brown seed coat color. Considering all these, the mutant was registered as cv. Binasola-7 for commercial cultivation in Barind (dry prone) areas in Bangladesh
Binasola-8	Bangladesh		Mutant cv, Binasola-8 was developed by hybridization with mutant cv. Hyprosola (released cultivar) obtained by irradiation of seeds with gamma rays (200 Gy), and one ICRISAT line K-850, made selections from F2 and onward generations. Main improved attributes are higher seed yield, medium seed size and attractive straw seed coat color. Considering all these, the mutant was registered as cv. Binasola-8 for commercial cultivation in Barind (dry prone) areas in Bangladesh
Binasola-9	Bangladesh		Main improved attributes are cream seed coat color (kabuli type), bolder seed size and higher seed yield
Binasola-10	Bangladesh		Main improved attributes are straw seed coat color, bolder seed size and higher seed yield

Source: Mutant Variety Database 2018 (https://nucleus.iaea.org/Pages/mvd.aspx)

complicated screening of elite genotypes and hence is very difficult to accomplish through conventional approaches (Torres 2009). This necessitates modern approaches of plant breeding.

3.6.1 Doubled-Haploid Production

The development of new and improved cultivars through conventional breeding approaches is time consuming and laborious. Anther culture represents an alternative approach and offers a quick method for recovering homozygous inbred lines. The main benefits of doubled-haploid production is the enhancement of cultivar improvement, increased homozygosity and response to market demands. But the success of anther embryogenesis and subsequent regeneration of complete haploid plants in Fabaceae is confined to a few species, like pigeon pea and alfalfa (Croser et al. 2006). The refractory nature of legumes makes progress in haploid plant production quite slow. The literature is scant on the protocols for haploid production in any cool season legume crop and only a little published literature is available (Altaf and Ahmad 1986; Croser 2002; Huda et al. 2001). Stress treatments such as heat or cold are frequently employed for anther or microspore culture of various crops like wheat (Gustafson et al. 1995; Touraev et al. 1996), mustard (Custers et al. 1994) and *Capsicum* (Kim 1999). The poineer work on the development of double-haploid chickpea cultivars such as the Canadian CDC Xena (kabuli) and the Australian

Sonali (desi) via anther culture using some physical stresses such as anther centrifugation and electrical shock, was put forth by Grewal et al. 2009. They developed a doubled-haploid method for two chickpea genotypes employing anther culture and combined stress treatments. Plants were raised inside phytotron chambers with controlled tempearture (22/12 °C) and photoperiods17/7 hat Saskatchewan University. After 15 days the seedlings were transplanted into plastic pots with 5 plants per pot. Plants were finally transferred to the greenhouse where additional lighting was provided through high-pressure sodium lamps. Flowers comprising various bud sizes (each containing10 anthers) were collected and exposed to a low temperature of about 4 °C for 4 days. 4′,6-diamidino-2-phenylindole (DAPI) stain was employed to screen the buds comprising of uninucleate microspores. Buds were sterilized with 20% buffered bleach for 15–20 min and washed3–4 times with autoclaved distilled water. Different buds were exposed to a range of individual and combined stresses to assess their effect on embryo development.

In the first group, flowers were subjected to cold temperature prior to anther inocculation on embryo development medium (Phillips and Collins 1979). In the second group, anthers were subjected to centrifugation at various rates (168, 377, 671 or 1509 g) for varying times 2, 3, 10 or 15 min. In the third group, anthers were exposed to electrical shock of 40–500 V. In the fourth group anthers were exposed to a combined stress treatments of lower treatment and centrifugation and lower treatment and electrical shock. A total of 90 anthers from treatment groups first to fourth were cultured on EDM for embryo induction and cultures were incubated at 24 ± 2 °C in darkness.

It was observed that lower treatment promoted embryo development in both cultivars. The cold stress stimulated enhancement in androgenesis has been attributed to the protection of microspores against the toxic compounds released in decaying anthers due to slowing of the degradation process in anther tissue, and hence facilitating the survival of more embryogenic pollen grains than in the heat treated anthers (Duncan and Heberle 1976). Amssa et al. (1980) also reported an increase in the endoreduplication of pollen at lower temperature leading to increased appearence in doubled-haploid plants. They attributed this to the alteration in several morphological and physiological processes and enzyme and hormonal balance in plant cells. In wheat, lower temperature leads to an abrupt increase in ABA content of anthers (Zur et al. 2008). The exposure of floral buds to cold treatment at 4 °C for 1 week enhances the androgenesis in various legumes including pigeon pea, grench bean and mung bean (Gosal and Bajaj 1988; Kaur and Bhalla 1998; Muñoz and Baudoin 2002); but, regeneration of haploid plants was obtained in pigeon pea at a low frequency.

Lower centrifugation rate and a shorter time period also led to early embryo formation and a greater number of anthers in comparison to control anthers. However, higher centrifugation rates and longer time intervals were detrimental to anther induction and decreased embryo formation. Mostly haploid embryogenic calli were induced with few doubled cells showing spontaneous doubling. Grewal

et al. (2009) attributed this to the spontaneous chromosome doubling occurring at the onset of regeneration in microspore or anther-derived callus. Similar spontaneous chromosome doubling has also been reported in several species; colchicine is not required to induce chromosome doubling to obtain doubled-haploid plants (Delaitre et al. 2001; Jain et al. 1996).

In both cultivars, an electrical current of 125 V led to high frequency of embryos per anther and quick embryo formation in comparison to the control. The low-voltage current of 50 V was ineffective in inducing the embryo formation while a higher electrical current range 250–500 V proved detrimental and hence decreased or lacked embryo formation due to major injuries to anther. In combined treatments anthers were subjected to lower speed centrifugation followed by the exposure of an 125 or 200 V electrical current and led to an increased rate of embryo formation. Delaitre et al. (2001) reported that application of an electric field to microspores of asparagus enhanced their androgenetic competence. The electric current of short-duration high-voltage pulses can also be used to enhance regeneration competence (Rech et al. 1987). Electrical currents create pores in cell membranes, thus facilitating transport of components across the membrane needed for cell development into the treated cells. Flow cytometric technique revealed haploid profiles and/or spontaneous doubling of the chromosomes during early regeneration stages of calli, embryos and regenerated plants.

Abdollahi and Rashidi (2018) were able to develop haploid plants from cultured anthers of two chickpea cvs., Bivanij and Arman, using high 2,4-D and silver nitrate containing media without applying any stress in the first experimentation. Several concentrations of 2, 4-D at a range of 0–10 mg/L) and silver nitrate concentrations of 0–50 mg/L were employed to stimulate androgenesis in embryo development medium. In cv. Bivanij, 10 mg/L 2,4-D and 15 mg/L silver nitrate were effective in inducing anther induction and production of embryos and regenerated plants per cultured anther; in cv. Arman, 10 mg/L 2,4-D in combination with the 15 and 50 mg/L silver nitrate produced the highest frequencies of embryos and regenerated plants. In a second experimentation, different cold treatment of 4 °C for 4–7 days and heat pretreatments of 30 °C for 10 days, 32 °C for 2 days and 35 °C for 8 h were applied on cultured anthers. Incubation of cultured anthers at 32 °C for 2 days considerably improved embryo formation, whereas cold treated anthers at 4 °C for 7 days and incubated at 30 °C for 10 days, induced maximum number of regenerated plants/anther. All the results pave the way for large-scale production of doubled-haploid chickpea plants.

3.6.2 Transgenic Approach

To maintain crop yield potential and address food security issues, crop production needs to be enhanced manifold times to feed the rapidly growing population. The desired goals of chickpea production have remained constantly low because of its susceptibility to a number of pathogens and insect pests. Among insect pests,

bruchids cause significant loss during storage (Singh et al. 1994). Conventional breeding methods have failed to create insect resistant cultivars and the direct treatment of seeds with chemical sprays is not recommended due to human consumption issues. Even though insecticides play a positive role in mitigating the impact of insect pests, they negatively affect the biota and their environment. Hence, to attain the desired production goal and to maintain a sustainable agriculture, new approaches for pest management are required to improve production, environment and health. Newer techniques like molecular breeding and genetic engineering need to be employed to develop insect resistant cultivars. Recently-developed recombinant DNA technologies and genetically-modified plants have proven successful for the management of pest and pathogen outbreaks (Gatehouse 2008). The insertion of foreign genes into the plant genome that confer resistance to insects have been made possible through advances in recombinant DNA technology (Bennett 1994). The δ-endotoxin genes, protease inhibitors and plant lectins are the main genes that were inserted and conferred a wide resistance to various pests and pathogens.

The combination of recombinant DNA technology and plant tissue culture has paved the way for the creation of novel options for biotic stress management, especially insect pests. These technologies have led to an immense reduction in the losses incurred by insect pests. Advances in biotechnology have created numerous distinctive opportunities such as plant transformation techniques, identification of novel and effective molecules and their role, alteration in gene expression and development of transgenics resistant to insect attack. In both developed and developing countries, transgenic plants have generated significant revenue. Special attention has been focusedon insecticidal*Bt* expressing transgenic plants that provide tolerance to herbicides (Shelton et al. 2002). Since their first introduction in the mid-1990s, the use of such crops has revolutionized agriculture and its allied sectors with input traits for pest management, primarily insects and herbicide resistance. Insect resistant transgenics play a critical role in the replacement of insecticides for pest management thereby reducing the pest-incurred yield losses. They also offer sources of resistance for deployment, which are otherwise unavailable from natural plant sources, and also help reduce huge investments in pest control over and above the basic requirements for raising a crop. This feature would be of matchless significance in enhancing the production of dry lands, if deployed effectively against insect pests of dry land crops.

Pests are responsible for huge production and yield losses in pulses including chickpea. Among them, the pod borer *Helicoverpa armigera* Hüberand the aphid *Aphis craccivora* C.L. Koch are the most damaging, causing an annual loss of USD 200 million in chickpea in India alone (Zahid et al. 2008). The development of transgenic chickpea lines showing resistance to *H. armigera* is considered as one of the best approaches to counter yield loss. Keeping these factors in mind, researchers at the University of Agricultural Sciences GKVK, Bangalore explored the possibility of developing insect resistant chickpea by overexpressing the synthetic *cry1X* gene against *H. armigera* (Asharani 2009). The study also facilitated abroader understanding of the development, evaluation and advancement of constitutively co-expressing *cry1X* gene in transgenic chickpea plant in the kabuli cv. KAK-2,

along with a kanamycin resistant marker gene, *nptII*. The synthetic gene *cry1X* harbors elements of the *cry1Aa, cry1Ab, cry1Ac* and *cry1F* genes. All these genes are basically active against Lepidoptera and have varied efficacy against different wide ranges of pests.

Researchers at Assam Agricultural University have developed transgenic chickpea lines showing resistance to pod borer infestation by expressing *Bt* genes (*Cry2Aa*). Sarmah et al. 2004 employed *Agrobacterium*-mediated chickpea transformation method leading to the development of the transgenic chickpea lines expressing high levels of a bean-amylase which acts as an inhibitor of porcine-amylase and led to improving resistance to *Callosobruchus* spp. *Bean AI1* was exclusively expressed in the seeds, accumulated up to 4.2% of seed protein and was processed to low molecular weight polypeptides as occurs in bean seeds. Insect resistant transgenic chickpea provide an exciting option as they are likely to reduce insecticide use for pest management and provide sources of resistance for deployment.

3.6.3 Tissue Culture

Conventional breeding approaches are time consuming and laborious for the development and official release of improved cultivars. This necessitates new modern breeding approaches such as tissue culture, plant regeneration strategies, gene transfer and plant transformation. Conventional breeding approaches like hybridization and induction of artificial mutations are based on the identification and enhancement of accessible genetic variability in the trait of interest. However, most pulse crops are self-pollinated which limits their genetic variability and due to this, plant breeders face the main problem of a narrow genetic base in crop improvement programs (Raina et al. 2016). Thus, it is imperative to widen the genetic base by the introduction of desirable genes from wild relative species into the cultivated species. In vitro culture techniques in crop improvement programs play a vital role in increasing the genetic variability and speed the process of conventional breeding. The recalcitrant nature of chickpea necessitates the development of more reliable tissue culture protocols to enhance genetic transformation and selection of stress-resistant plants (Ochatt et al. 2010). Kadiri et al. (2014) researched suitable conditions for chickpea in vitro micropropagation using mature embryos and nodes as explants. Three chickpea genotypes (Zouaoui, ILC 483, INRA 199) were used as a source of explant cultures on Murashige and Skoog (1962) medium containing naphtyl acetic acid (NAA), Benzyl amino purine (BAP) and kinetin (KIN). Zouaoui genotype was found to be more callogenic than INRA 199 and ILC 483. It was observed that they are less oriented to callogenesis than cells dedifferentiation or pre-existing meristems development. Various factors such as genotype, explant and nutrient medium influence the callogenesis, cell dedifferentiation and regeneration (Yadav et al. 2012). They also noted that Zouaoui, ILC 483 and INRA 199 exhibited organogenesis capacity at 52.73, 59.76 and 47.50%, respectively.

The type and the concentration of exogenously applied hormones influence behavior of in vitro cultured explant. The choice of hormones in tissue culture is made according to the targeted morphogenetic response and the type of explants (Altaf et al. 1999). The main hormones such as auxins and cytokinins are employed to achieve callogenesis or organogenesis, acting in synergy or antagonism (Zryd 1988). Irrespective of the type of explants employed, MS culture media enriched with added hormones showed differential rates of organogenesis and callogenesis. On the other hand, 76.16% of cultured explants expressed their organogenic ability in MS medium without added hormones. Kilikova et al. (2004) reported that the addition of both auxin and cytokinin in M14, explants promoted more callus formation and hence concluded that endogenous hormones influence the seedling in vitro development. Comparable results were reported for in vitro culture of different chickpea explants (Aasim et al. 2011; Huda et al. 2003; Sagare et al. 1993). For direct organogenesis or callogenesis embryonic axes are proffered as they are more reactive than nodes. Additionally, calli on the same fresh media grew differently and only those formed on MS supplemented with 0.5 mg/L KIN revealed formation of buds and prominent leaf primordia. Indirect regeneration of chickpea is dependent on the addition of optimal low concentration of exogenous cytokinin hormone (Arora and Chawla 2005; Weerakoon 2010). MS medium supplemented with 1 mg/L KIN was found to be unfavorable for formation of buds in a genotype independent manner. Kumar et al. (2013a, b) report that this finding is of paramount significance in genetic transformation where nongenetic dependent regenerations are needed to curtail phenotypic aberrations and cytogenetic alteration.

Ghanti et al. 2010 developed a protocol for plant regeneration in chickpea cvs. ICCV-10 and Annigeri using somatic embryogenesis. Immature cotyledons were used as explants on MS medium supplemented with varied concentrations of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA) and picloram, alone or in combination with N6-benzylaminopurine (BA) or kinetin to facilitate formation of somatic embryos. Somatic embryos were induced at the highest frequency in NAA than other auxins. The MS medium supplemented with 2 mg dm-3 BA + 0.5 mg dm-3 ABA (abscisic acid) facilitated the transformation of cotyledonary-shaped embryos into plantlets with a frequency of about 36.6 %. The cv. ICCV-10 showed higher frequency of embryogenesis and plantlet regeneration than cv. Annigeri. Histological studies of explants at different developmental stages revealed the direct development of somatic embryos from the cotyledon cells. The developing embryos were highly ordered, round, creamish colored that arise from the surface of cotyledons when cultured on MS medium supplemented with varied concentrations of 2,4-D or 2,4,5-T or NAA or picloram. However, MS basal medium without hormonal supplementation resulted in less or no embryogenesis. Embryogenesis was higher in the presence of NAA followed by 2,4,5-T, 2,4-D and picloram. The highest frequency of embryogenesis was recorded at a concentration of 6 mg/L NAA. But the NAA concentration beyond 6 mg/L reduced both the frequency and number of somatic embryos. These results closely match previous findings in soybean, *Glycine max* (L.) Merr. (Lazzeri et al. 1990), black gram, *Vigna mungo* (L.) Hepper (Eapen

and George 1990), ajwin, *Trachyspermum ammi* (L.) Sprague ex Terrill (Sehgal and Abbas 1994), mung bean, *Vigna radiate* (L.) R. Wilczek (Girija et al. 2000) and castor bean *Ricinus communis* L. (Ganesh et al. 2008). The results were in disagreement with the findings of Sagare et al. (1993) in chickpea. They documented the highest frequency of somatic embryos from immature cotyledon derived callus. Similarly, Barna and Wakhulu (1995) obtained somatic embryos from leaf callus of chickpea. The development of somatic embryos through the callus phase can lead to wide somaclonal variation, while direct regeneration avoids it. The cv. ICCV-10 produced more embryos as compared to cv. Annigeri, reflecting genotype dependent embryogenesis, as earlier reported in other legumes (Ozias et al. 1992, Sagare et al. 1993, Venu et al. 1999). Ghanti et al. 2010 concluded somatic embryos were directly induced from immature cotyledon without an intervening callus stage and the number of somatic embryos per explants was high. Based on this study it can be concluded that in chickpea tissue culture morphogenetic response is dependent on the choice of genotype and culture medium. Among media, MS without hormones, stimulated development of preexisting meristems while as MS with NAA/BAP was suitable for callogenesis.

Rao and Chopra (1989) also obtained somatic embryos using leaflet-derived callus of chickpeaas explants grown on MS medium supplemented with hormone incubation conditions. MS medium supplemented with 0.5 mg/L each of 2,4-D and BAP and dark incubation was found best for producing embryogenic callus. Callus induction was apparent from the cut surface of the explants within 1 week of incubation followed by profuse callusing along the leaf margin and vein sunder both dark and light incubations. The frequency of embryogenesis was greatly influenced by the hormonal concentration in the culture medium and it was highest at 0.5 mg/L 2,4-D and 0.5 mg/L BAP. The combination of hormones in the medium on which embryogenic response was tested has a significant influence. The 2,4-D plays a critical role in regulation of embryogenic development, an increase in its concentration leads to a decrease in number of embryoids and its withdrawal leads to embryoid induction (Kohlenbach 1977).

Ugandhar et al. 2012 developed a quick, easy and proficient procedure for in vitro multiple shoot induction and plantlet regeneration using shoot tips and cotyledonary nodes as explants of *Cicer arietinum* cv. ICCCR (kranthi), cultured on MS medium supplemented with BAP 0.5–3 mg/L and KIN 0.5–3 mg/L. Multiple shoot proliferation was best observed in BAP at a concentration of 2 mg/L and hence it was found to be more effective than KIN for shoot multiplication. The highest number of shoots was achieved on MS medium fortified with 2 mg/L BAP. The medium supplemented with 2 mg/L BAP proved better than all other media concentrations in cotyledonary node explants. Shoot tip explants were stimulated for the induction of multiple shoot at different concentrations of BAP. It was found that a concentration of 2 mg/L BAP was more effective in inducing maximum shoots/explants. However, with the increase in concentration of BAP beyond 3 mg/L, there was a progressive decrease in the number of shoots/explants. Shoot tip explants were capable of directly developing multiple shoots on MS basal medium fortified with varied concentrations of KIN in the range of 0.5–3 mg/L. Maximum number of shoots was found at 2 mg/L

of KIN. Recently, shoot tip explants have received much attention to produce huge numbers of genetically similar clones. In faba bean, multiple shoots were borne from shoot apices using MS medium supplemented with 20 μM BA, 0.1 μM NAA (Griga et al. 1986). The combination of BAP and KIN enhanced the regeneration potential of shoot apical meristems of various crops such as soybean, peanut, cowpea, bean and chickpea. It was concluded that BAP is an ideal hormone for shoot multiplication of shoot tip culture in legumes (Sounder et al. 1989). These findings were inline with those on teak, *Tectona grandis* L. f. (Gupta et al. 1980) and the lebbeck tree, *Abizzia lebbeck* (L.) Benth (Gharyl and Maheswari 1982); multiple shoot induction was also observed in jujube, *Ziziphus manritiana* Lam. (Sudharshan et al. 2000) and vanilla, *Vanilla plantifolia* Jacks. ex Andrews (Geetha et al. 1999), with shoot tips cultured on MS + cytokinin alone. Ugandhar et al. 2012 documented that any variation in the hormonal concentration influences shoot bud differentiation and shoot proliferation from shoot tip explants of *Cicer arietinum* cv. ICCC (kranthi). Moreover, cytokinin facilitated shoot bud induction and proliferation and its absence in the basal medium led to no shoot bud induction and proliferation. Similar results are well documented in several medicinal plants (Pattnaik and Chand 1996) such as amla, *Emblila officinale* Gaertn. (Verma and Kant 1996) and ashwagandha, *Withania somnifera* (L.) Dunal (Deka et al. 1999). Ugandhar et al. 2012 concluded that 2 mg/L BAP and KIN were significantly more effective for inducing shoot organogenesis. It is evident that BAP and KIN are the best suited for inducing multiple shoots.

One of the most effective procedures of micropropagation in plants is cotyledonary node induction as the buds borne from meristematic tissue offer huge potential for vigorous development (Yadav et al. 1990). Axillary buds have been reported as the suitable explant for clonal propagation in various crops like the Indian bdellium tree, *Commiphora wightii* (Arn.) Bhandari (Barve and Mehta 1993); black mulberry, *Morus nigra* L. (Yadav et al. 1990) and holy basil, *Ocimum sanctum* L. (Shahzad and Sidique 2000). Cotyledonary node explants of chickpea cultured on varied hormonal combination revealed different results. In the first week of inoculation, the axillary buds become active and gave rise to new shoots with leaves and internodes in the subsequent second and third week. It was found that the cotyledonary node size plays a crucial role in initiation and also promotes elongation of shoots. The smaller explants can initiate more multiples than the longer cotyledonary node explants. The maximum number of shoots/explant was achieved in a medium containing 1 mg/L BAP. A progressive decrease was observed in shoot bud proliferation, rate of shoot multiplication and elongation with the increase in the concentration of BAP beyond 1 mg/L. The healthy elongated shoots were transferred on to one-half strength MS root induction medium (RIM) (Murashige and Skoog 1962) supplemented with varied concentrations of IAA in the range of 0.5–2 mg/Land IBA in the range of 0.5–2 mg/L. The concentrations 1.5 mg/L of IAA and 1 mg/L IBA were found to facilitate profuse rhizogenesis. From the above study, it was concluded that shoot tip and cotyledonary node explants are suitable for clonal propagation of chickpea. Cotyledonary node explants may be used for their higher rate of shoot multiplication. The protocol described in the present study is reproducible and can be used in future for further developments of the crop.

3.6.4 Genetic Mapping

Modern breeding approaches effectively use genomic resources by mapping the markers associated with particular traits. Construction of genetic maps is a primary step in the linkage mapping-based identification of markers associated with particular traits. In the past few years several molecular markers and genetic maps have been developed. These approaches have established an association between genes and phenotypic variation in both qualitative and quantitative traits. Quantitative traits are governed by small additive effects of multiple genes and are also influenced by environmental flux to a great extent. As a result breeding for such polygenic traits is more complex as compared to qualitative traits. The breeding behavior of quantitative traits can be studied through the construction of genetic maps, as the maps focus on a single region known as quantitative trait loci (QTL), responsible for phenotypic variation of a particular trait. Due to limitations of employing morphological markers in the development of genetic maps, several molecular markers such as diversity arrays technology (DArT) and single nucleotide polymorphisms (SNPs) were found effective. A genetic map with more than 1200 loci covering a distance of 845 cM on 8 linkage groups has been reported in an interspecific mapping population (ICC 4958 x PI 489777) of chickpea (Thudi et al. 2011). Varshney et al. (2014) while working on the drought tolerance, developed a genetic map comprising 241 loci and 168 loci in 2 intraspecific mapping populations ICC 4958 x ICC 1882 and ICC 283 x ICC 8261, respectively. A fine genetic map with more than 1000 marker loci spanning a distance of 727 cM was developed through recently developed the genotyping-by-sequencing (GBS) approach used by Jaganathan et al. (2015) in the same mapping populations, ICC 4958 x ICC 1882. Additionally, several researchers have also developed different transcript maps in chickpea. Gujaria et al. (2011) worked on an interspecific mapping population (ICC 4958 x PI 489777) and constructed a transcript map with molecular markers like SNP, SSR and intron spanning region (ISR) markers. Hiremath et al. (2012) developed a second-generation transcript map comprising 1328 marker loci with a mean inter-marker distance of 0.59 cM. Identification of 312 markers associated with drought and heat response in association mapping analysis using whole genome scanning and candidate gene-based approach has been reported in chickpea (Thudi et al. 2014). Successful genetic mapping and QTL analysis, not only in chickpea but in other legumes, also have been facilitated by the availability of a huge number of DNA markers. Genetic maps play a vital role in dissecting the complex traits, particularly yield and yield attributing traits.

The advent of high throughput genomic resources has played a critical role genetic enhancement through improvement in yield, nutritional quality and tolerance to wide range of biotic and abiotic stresses in chickpea. The quick advancement in the chickpea genomics is reflected in the development of a huge number of molecular markers for assessment of genetic variation. At the outset SSR markers were used by several workers for diversity assesment (Sethy et al. 2006) identification of QTLs (Aryamanesh et al. 2010) and development of genetic maps (Nayak et al. 2010). However, very recently a

large scale discovery and genotyping of SNPs have been the major advancements in chickpea (Deokar et al. 2014). These advancements led to the official release of the draft genome sequences of two major chickpea types i.e. desi [*Cicer arietinum* ICC4958] (Jain et al. 2013) and kabuli (*C. arietinum* CDC Frontier) (Varshney et al. 2013b). Gaur et al. 2015 carried out the genome-wide discovery of SNPs through next generation sequencing of the genome of *Cicer reticulatum*. They carried out a study which was aimed at large scale discovery of SNPs from the genome sequence of the wild species *C. reticulatum* PI489777 and the cultivated *C. arietinum* ICC4958, parents of the reference mapping population. A most sophisticated high-density linkage map of chickpea with wide genome coverage and marker density was constructed by employing the SNP resources. The genetic linkage map of chickpea was constructed by using 5013 polymorphic SNPs1065 markers from the earlier study (Gaur et al. 2012) and 636 SSR markers from Khajuria et al. 2015 using Illumina GoldenGate technology. The genotyping data of 6714 markers across 129 RILs involving 866,106 data points were employed to construct the linkage map. The map positions of 6698 markers scattered on 8 linkage groups designated as CaLG1 to CaLG8 were defined on the constructed map (Fig. 3.7). The average inter-marker distance of 0.16 cM with total span of 1083.93 cM was recorded. The map contained an average of 9.05 map positions per Mbp of genome and represented an average physical interval of 110.48 kb/marker. The genetic length of the LGs ranged from 98.798 cM (CaLG1) to163.633 cM (CaLG8). The CaLG1, CaLG3, CaLG4, CaLG5, CaLG6 and CaLG7 were highly saturated with less than 0.2 cM average marker density and 778 to 1050 markers. Hence, one linkage group harboured 837.25 markers spanned on 135.49 cM of genetic length (Fig. 3.7). The constructed genetic map is the most dense linkage map of chickpea, with the potential to assist efficient anchoring of the draft genome sequences of chickpea varieties.

Khajuria et al. 2015 designed worked out 1494 markers, comprising of 1016 genomic and 478 transcript-derived microsatellite markers showing in-silico fragment length polymorphism between two inter-specific reference mapping population (parental genotypes *Cicer arietinum* ICC4958 and *C. reticulatum* PI489777). The genotyping of 94 individuals of RIL mapping population was carried out by employing 636 novel (Gaur et al. 2015) microsatellite markers including 175 genomic and 461 transcript-derived microsatellite markers revealing parental polymorphism between ICC4958 and PI489777 for construction of inter-specific genetic linkage map. An advanced, high-density, integrated and inter-specific chickpea genetic map (ICC4958 x PI489777) having 1697 map positions spanning 1061.16 cM with an average inter-marker distance of 0.625 cM was constructed by assigning 634 novel informative transcript-derived and genomic microsatellite markers on eight linkage groups (LGs) of our prior documented, 1063 marker-based genetic map. Similar studies have also been reported by Thudi et al. (2011) in which the genetic maps spanning 845.56 cM with mean inter-marker distance of 0.65 were created employing 1291 microsatellite and Hiremath et al. (2012) constructed genetic map spanning 788.6 cM using 1328 SNP and DArT (diversity array technology) markers with mean inter-marker distance of 0.59 cM.

Nonetheless, all of these genetic maps constructed were based on 300 microsatellite markers and RAPDs, ISSRs (inter simple sequence repeats), CAPS (cleaved

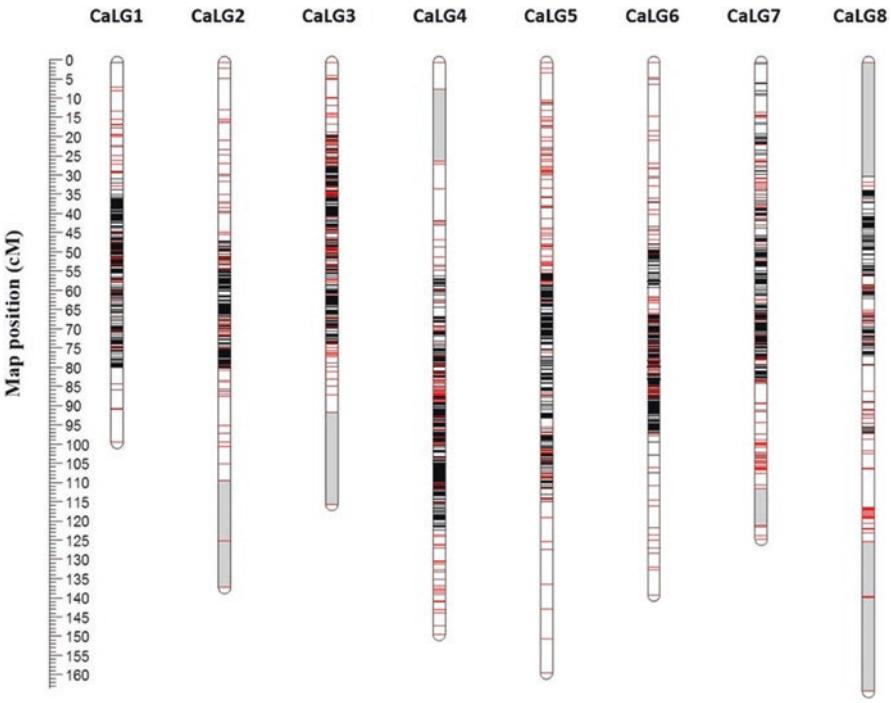

Fig. 3.7 High-density genetic linkage map of chickpea. The inter-specific linkage map of chickpea based on RILs of *C. arietinum* (ICC4958) × *C. reticulatum* (PI489777) harbouring 6698 loci. The name of the linkage groups is mentioned at the top of each LG. SNP markers are represented in black, while red colour is shown for markers other than SNPs. Large gaps with > 10 cM length were observed at the proximal offends of different LGs (CaLG2, CaLG3, CaLG4, CaLG7 and CaLG8) and are represented in grey shade. (Source: Gaur et al. 2015. Creative Commons license http://creativecommons.org/licenses/by/4.0/)

amplified polymorphisms), intron-spanning markers, SNPs and DArT markers. In contrast, Khajuria et al. 2015 constructed high-density genetic linkage map, and were able to assign 634 novel, codominant, sequence-based genomic and transcript-derived microsatellite markers, which is much higher than the number of microsatellite markers (~300 markers) mapped until date in chickpea. The genetic map had a very high map density (0.625 cM) and thus was an advanced, highly saturated chickpea genetic map in comparison to all other intra- and inter-specific genetic linkage maps reported so far in chickpea. The LGs were numbered with Arabic numerals ranging from LG1 to LG8 according to their common marker positions and groupings shared between corresponding LGs as reported by earlier studies (Winter et al. 2000; Nayak et al. 2010; Gujaria et al. 2011; Thudi et al. 2011). The highest number of markers were mapped on LG3 (376) followed by LG5 (276) and LG4 (251) and least on LG2 (105). This sophisticated high-density map will pave a way for establishment of genotyping, large-scale marker validation, identification and mapping of trait-specific genes/QTLs with sub-optimal utilization of resources and labour in chickpea.

Considering the desirable genetic attributes of microsatellite markers, hence, the microsatellite marker-based integrated, high density and inter-specific genetic linkage map would be useful for mapping the whole genome and rapid targeted mapping of genes/QTLs controlling useful agronomic traits in chickpea as well as comparative mapping across legumes.

3.6.5 Marker-Assisted Selection

The use of marker-assisted selection (MAS) in crop improvement programs have increased in recent years. The main advantage of MAS over conventional plant breeding is the reduced number of generations and the population size required to release elite cultivars (Castro et al. 2015; Thomas 2003; Yousef and Juvik 2001).

The MAS approach is not only used for accelerating the pace of breeding programs, but has also led to the establishment of gene pyramiding which allows combining desirable QTLs from multiple parents to develop elite cultivars. A successful implementation of MAS in a breeding program requires the appropriate choice of genotype and subsequent phenotypic selection of candidate genes and their associated markers. Genotypes are chosen on the basis of the presence or absence of markers and not the associated trait. Simon and Muehlbauer (1997) used RFLP and RAPD markers in the construction of a chickpea map and reported a synthenic relationships with other legumes. Simple sequence repeats (SSRs) or microsatellites have vast distribution in coding and non-coding sequence as tandem repeats of 1-6 DNA nucleotide motifs (Varshney et al. 2005a, b; Parida et al. 2009). Microsatellites are one of the hypervariable class of PCR based genetic markers as any variation in the number and/or size of microsatellite repeats at a locus among individuals results in different sized DNA bands (Beckmann and Soller 1990). Microsatellite markers are very informative and have gained substanable significance over other molecular markers for assessment of genetic diversity in crop plants because of their multiallelic nature, co-dominant inheritance, reproducibility, abundance and wide genomic distribution. SSRs are also amenable to large-scale genotyping and hence suitable for construction of high-density genome maps, gene/QTL mapping and marker-assisted selection (Winter et al. 2000; Nayak et al. 2010; Gujaria et al. 2011).

In chickpea, the presently available draft genome sequences of desi cultivars generated about 30000 microsatellites and 81000 microsatellites in kabuli cultivars in silico (Jain et al. 2013; Varshney et al. 2013b). The validation of such massive numbers of microsatellite markers and screening of informative markers that reveal successful amplification as well as required polymorphism in chickpea, is an enormously laborious and tiresome task. Furthermore, because of the narrow genetic base and lesser intraspecific polymorphism among chickpea genotypes, it is difficult to employ a larger proportion (~70%) of the identified markers (Choudhary et al. 2012; Gujaria et al. 2011; Nayak et al. 2010). Moreover, the screening and selecting of the remaining ~30% informative polymorphic microsatellite markers

from the huge microsatellite marker database, is very costly, laborious, cumbersome process. In order to obtain the desired polymorphic potential and for enriching the informative-ness of microsatellite markers, the workers also need sophisticated infrastructural amenities such as high-resolution genotyping assays for accurate marker allele sizing and estimating their accurate intra-specific allelic variations among chickpea genotypes. To overcome the limitations in silico analysis could be employed for validation and genotyping of the great number of microsatellite markers at individual and genome level. Recently efforts have also been put forth to identify in silico polymorphic microsatellites by compereative analysis of the whole genome and transcript sequences among kabuli, desi and wild chickpea genotypes (Jain et al. 2013; Agarwal et al. 2012; Jhanwar et al. 2012). Single nucleotide polymorphisms (SNPs) with maximum abundance are considered ideal molecular markers. The creation of high yielding varieties that can withstand environment induced abiotic and biotic stresses has been the area of focus for many researchers in the past. Plant breeders employ conventional breeding approaches to overcome the production constraints. The success of creating elite varieties in many crops have been done genomics-assisted breeding (GAB) (Varshney et al. 2005a, b, 2010). However, due to scanty genomic resources of chickpea GAB applications have been restricted and suitable mapping populations are required to understand the genetics of complex traits and limited genetic diversity in superior germplasm (Varshney et al. 2010).

Several evolutionary bottlenecks lead limited genetic diversity of chickpea have been documented and the use of wild species to enhance the genetic diversity in primary gene pool are recommended (Abbo et al. 2003). However, noteworthy advancements have been achieved in the field of developing genomic resources in these legume crops (Varshney et al. 2013a) including thousands of simple sequence repeats(SSRs) (Thudi et al. 2011), single feature polymorphism (Saxena et al. 2011), single nucleotide polymorphism (SNP) (Saxena et al. 2012), and diversity arrays technology (Thudi et al. 2011)and expressed sequence tags (ESTs) (Kudapa et al. 2012) were developed. Very recently, draft genome sequences have also become available for chickpea (Varshney et al. 2013b). Based on huge genomic resources now available, GAB approaches can be employed to complement the traditional breeding approaches in chickpea.

Even though a huge number of molecular marker systems have been developed, SNPs are preferred markers for genetics and breeding applications (Mir and Varshney 2013). Recent progress in next generation sequencing technologies complemented with bioinformatics tools led to the cost-effective discovery of SNPs and their ensuing utilization in genome analysis and crop improvement (Thudi et al. 2012). In chickpea thousands of high confidence SNPs have been developed (Hiremath et al. 2012) and the choice for SNP genotyping platforms are also huge (Varshney 2011) for studying the genetic aspects but the selection of platform depends objectives, sample size and markers to be analyzed. Array-based genotyping systems such as the Illumina GoldenGate (Illumina, Inc.) and Infinium (Illumina, Inc.) are now available, of which Illumina GoldenGate has regularly was used for mid-throughput applications (Fan et al. 2003). Roorkiwal et al. 2013 designed and

tested 96 SNPs by genotyping 288 diverse chickpea genotypes with the objective to create cost-effective SNP genotyping platforms in chickpea. The SNPs selected for the oligo pool assays had high transferability to crop wild relative species. The selected SNPs revealed polymorphism between the parental genotypes of 14 mapping populations of chickpea from chickpea reference set (Upadhyaya et al. 2008). A highest of 29 SNPs were common between any two populations, while 19 and 14 SNPs were common when comparing any three or four mapping populations respectively. The mean PIC value of SNP markers was 0.31 and the majority of SNPs were highly polymorphic with PIC value ranging from 0.3 to 0.4 whereas less than 5% of the SNPs were found to have PIC value less than 0.1. Gene diversity across chickpea reference set varied from 0.01 to 0.5, with a mean value of 0.40. The neighbor joining tree based genetic relationships in reference sets grouped chickpea genotypes into four major clusters (Cl). The Cl I contained 74 genotypes of Indian origin, which include four wild, 20 kabuli, and three pea-shaped genotypes. The Cl II, Cl III, and Cl IV contained 52, 38, and 124 genotypes, respectively. Simple sequence repeat–based dendrograms of chickpea and pigeonpea reference sets obtained in earlier studies (Upadhyaya et al. 2008) were compared with the SNP-based dendrograms developed in this study. In the case of chickpea, 36 SSR markers grouped the reference set into two major clusters while SNP markers demarcated the genotypes into four clusters. Despite having enough diversity, no clear group based on biological, geographical, or seed type could be evident when comparing the dendrograms. The Illumina BeadXpress platform assays developed for chickpea are highly informative and cost effective for undertaking genetic studies in these legume species. The screening of diverse reference set and parents of mapping population confirms that such assays can be used to characterize diverse germplasm and also to integrate more markers into existing genetic maps.

Recently, various molecular markers with the ability to produce polymorphisms from genic regions of the genotype have been created. They include SRAP (sequence related amplified polymorphism: Li and Quiros 2001); TRAP (target region amplification polymorphism: Hu and Vick 2003); SCoT (start codon targeted polymorphism: Collard and Mackill 2009); CoRAP (conserved region amplification polymorphism: Wang et al. 2009) and CBDP (CAAT box derived polymorphism: Singh et al. 2014a, b, c). The SCoT polymorphism is a simple and dependable gene targeted marker technique based on the conserved region surrounding the translation codon ATG has been used in *Cicer* for genetic diversity analysis (Collard and Mackill 2009). Another gene targeted marker, CBDP (CAAT box-derived polymorphism) is dependent on the CAAT box region of promoters in plant genes (Singh et al. 2014a, b, c). With the advent of molecular markers and accessibility of genomic resources, strong associations between markers and traits can be easily identified. A huge number of genomic resources were deployed in chickpea for the screening and isolation of genes/QTLs governing qualitative and quantitative characters. Some of them are early-flowering genes (Gaur et al. 2016; Mallikarjuna et al. 2017); drought responsegenes (Chandra et al. 2004; Varshney et al. 2014); *Ascochyta* blight resistance (Aryamanesh et al. 2010; Varshney et al. 2014); *Fusarium* wilt resistance (Tekeoglu et al. 2000) and *Botrytis* gray mold resistance (Anuradha et al. 2011).

After identification, these QTLs were employed for improvement in developing resistance against several kinds of biotic and abiotic stresses.

3.6.6 Marker-Assisted Recurrent Selection (MARS)

In marker-assisted recurrent selection (MARS) molecular markers are employed for the identification and selection of multiple QTLs to develop elite genotypes within a single or across related populations (Ribaut et al. 2010). MARS provides a quick method of raising generations as it involves individual genotypic selection and intercrossing in one cycle of selection. This advanced molecular breeding approach is quite different from traditional QTL or MAS studies as it involves a new mapping study on each breeding population and increases desirable allele frequency in the populations. Several workers advocated that MARS improves the selection process and plays a role in combining different desirable genes in a genotype by employing multi-parent population and recurrent selection. A new mapping study is conducted on each breeding population in this molecular breeding scheme that distinguishes it from traditional QTL or MAS studies. In commercial breeding of crops like maize, soybean and sunflower, MARS has proven effective in improving yield and yield attributes and increasing genetic gains (Johnson 2003). Drought tolerant chickpea genotypes were developed at ICRISAT by conducting MARS on four desi genotypes (ICCV 04112, ICCV 05107, ICCV 93954, ICCV 94954). On the basis of F3 genotyping and F5 phenotyping data (from Ethiopia, Kenya and India) many agro-economical QTLs were identified. In order to integrate desirable alleles, two crosses were made by using elite lines JG 11 ICCV 04112 and JG 130 ICCV 05107, each having a set of eight lines which were selected for each cross using OptiMAS ver. 1.0 (Valente et al. 2013). It is expected that the completion of the project will make available the RC3F4 progenies for multi-site evaluation. Efforts have been initiated to employ MARS in chickpea to assemble desirable alleles for drought tolerance using ICCV 04112 ICCV 93954 and ICCV 05107 ICCV 94954 crosses. These efforts will result in the creation of cultivars with increased drought tolerance. The Indian Agricultural Research Institute (IARI) and Indian Institute of Pulse Research (IIPR) have also begun MARS research in chickpea by employing Pusa 372 JG 130 and DCP 92–3 ICCV 10 crosses. These research collaborations will lead to the development of superior lines with enhanced drought tolerance and wide adaptability. In short, MARS is a modern breeding approach that equips plant breeders with increased frequency of desirable alleles and with an additive effect and small individual effects in recurrent crosses (Bernardo and Charcosset 2006).

3.6.7 *Multi-parent Advanced Generation Intercrossing (MAGIC)*

The degree of available and accessible genetic variability is imperative in developing tolerance against the wide range of stresses. MAGIC, an advanced breeding approach to equip plant breeders with a high degree of genetic variability and high frequency of recombinants can also be used to understand the underlying mechanism polygenes that govern the quantitative traits (Glaszmann et al. 2010). Development of MAGIC lines provides a basis to analyze the segregation among several QTLs that govern agro-economical traits. Conventional breeding such as hybridization may limit the productivity by creation of cultivars with desirable alleles in a homozygous condition. Unlike conventional hybridization, MAGIC populations are very useful in the accurate detection of QTLs, gene discovery, gene characterization and understanding of molecular characterization of complex traits (Buckler et al. 2009; Poland et al. 2011). In South Asia and Sub-Saharan Africa, eight chickpea parents were employed to create MAGIC lines with the aim of improving adaptability potential to diverse agro-ecosystems. The chickpea MAGIC lines were also used in the identification of several QTLs with high precision. Different hybridization techniques used by plant breeders have enhanced genetic variability for economically important traits and additionally it will also expose the rare alleles in homozygous condition.

Based on this, MAGIC lines were created in chickpea by employing 8 diverse genotypes collected from South Asia and Sub-Saharan Africa. ICRISAT developed the F4 MAGIC population by employing 28 two-way, 14 four-way and 7 eight-way crosses with parents ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108, collected from Ethiopia, Kenya and India. Attri et al. (2018) collected the F4 MAGIC lines from ICRISAT and raised the F5 generation in the winter (*Rabi*) season of 2013–2014. The progenies were found to have high yield and tolerance to drought. Seed yield and pod per plant exhibited very high heritability along with high genetic advance. Significant differences existed for all the quantitative traits in the segregating generations, suggesting that the genetic improvement in the segregating generations were of a high degree, which can be further utilized in chickpea breeding.

3.7 Conclusions and Prospects

The main objectives of a breeding program are recognizing the desired genetic variability for agro-economical traits, utilizing that variability and propagating the desired populations to future generations. Although traditional breeding methods are useful to exploit the accessible genetic variability in the cultivated germplasm,

which has led to the development of several high yielding and better adaptable cultivars of chickpea, the key limitation is the extended time period required for the procedure. In recent years, several genetic maps have been developed and important QTLs dissected for traits of interest in chickpea. New genomic advances, several of which are already being developed, will equip plant breeders to create new cultivars with superior characteristics, facilitating selection and improving the variation. In particular, the present and new genomics tools add great value in the process of genetic dissection and breeding of complex traits. Until now, genomic tools played a crucial role in QTL identification, and their use in chickpea breeding was limited to improving tolerance to a wide range of biotic and abiotic stresses. Yield is highly dependent on the environment such as soil, water and cultivation conditions; however, the effects of climate change, along with lessening arable land, water resources and erratic rainfall, have major impacts on overall chickpea production. There is a need to create cultivars which can tolerate climate change and to adopt appropriate chickpea cultivars fitting the environment to enhance their productivity.

Appendices

Appendix I: Research Institutes Relevant to Chickpea

Institution	Specialization	Contact information
Akdeniz Üniversitesi, Akademik Veri Yönetim, Sistemi, Turkey	Plant genetic resources, pre-breeding and phenotyping of plants under stress conditions such as cold, drought, salinity, *Ascochyta* blight, leaf miner and seed beetles	Dr. Cengiz Toker http://aves.akdeniz.edu.tr/
International Crops Research Institute for the Semi-Arid Tropics(ICRISAT), Hyderabad, India	Applied genomics, molecular breeding, comparative and functional genomics and crop biotechnology	Dr. Rajeev K Varshney https://www.icrisat.org/
Indian Institute of Pulses Research(IIPR), Kanpur, India	Breeding	Dr. Yogesh Kumar iipr.res.in
Indian Institute of Pulses ResearchIIPR, Kanpur, India	Breeding, abiotic stress	Uday Chand Jha iipr.res.in

Appendix II: Chickpea Genetic Resources

Cultivar	Important traits	Cultivation location
DCP 92-3	Tolerant to lodging, wilt resistant, yellowish small seeds	North-Western Plains Zone, India
IPC 97-67 (SCS-3)	Early maturing, resistant to wilt and tolerant to terminal moisture stress	Jammu, India
IPCK 2002-29 (Shubhra)	Kabuli chickpea variety, large seeds (34 g/100-seed wt.), moderately resistant to wilt	Central Zone India
IPCK 2004-29 (Ujjawal)	Moderately resistant to fusarium wilt	Central Zone India
Venhar	Desi, high yielding, medium seeded, tolerant to A blight, suitable for cultivation in Pothwar region	BARI, Chakwal, Pakistan
Dashat	Desi, high yielding, medium seeded, resistant to *Ascochyta* blight, suitable for cultivation in Pothwar region	NARC, Islamabad
Parbat	Desi, high yielder than Dasht medium seeded, resistant to *Ascochyta* blight, suitable for cultivation in Pothwar region	NARC, Islamabad
BARI Chola-10	heat-tolerant, resistant to *Botrytis* gray mold (BGM) and also high-yielding	Ishurdi, Gazipur, Madaripur, Barishal, Jessore and Rajshahi districts of Bangladesh
Binasola-10	Binasola-10 is a high yielding chickpea variety, released in 2016. It matures in 115–122 days, hundred seed weight is 23.5g	Bangladesh
Binasola-6	Binasola-6 is a high yielding chickpea variety, released in 2009. Plant height varies from 48–60 cm. Maturity period ranges between 122–126 days. Maximum yield potential is 1.97 m/ha (av. 1.69 mt/ha). It has bright seed coat color. Seed contain 23.10 % protein	Bangladesh

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Chapter 4
Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub] Breeding

Aravind Kumar Jukanti, Rakesh Pathak, and Chiranjeevi Mushyam

Abstract Cluster bean is an important leguminous annual crop of arid and semi-arid regions of northwestern India, mainly in Rajasthan and parts of Gujarat, Haryana and Punjab. India contributes about 80% of the global cluster bean production. It is a source of gum (guar gum/galactomannan), a natural hydrocolloid having unique qualities and wide applicability. It is a highly self-pollinated crop with very limited outcrossing. Limited genetic diversity for the different traits coupled with yield losses caused by different biotic and abiotic stresses have constrained intensive breeding efforts of this crop. Despite these limitations, progress has been achieved by exploiting the available diversity and different breeding methods like pedigree selection in the cluster bean improvement program with good results. Hybridization has not been successful due to the small, delicate flower resulting in a low percentage of hybrid seed setting. Mutation breeding has helped to generate some genetic variability in certain important traits, but it has had limited impact on cluster-bean breeding. DNA-based molecular markers are being extensively used in genetic diversity analysis and phylogenetic studies. Efforts are also underway to develop genomic resources (SSR and SNP markers) that could be used in cluster-bean breeding. Limited transcriptomic and mi-RNA studies have also been recently reported. With the availability of advanced molecular tools it would be feasible to develop resources to aid in high-yielding, cluster-bean varieties with moderate to high gum content for a much preferred export commodity.

Keywords Botany · Breeding methods · Cluster bean · Genetic variability · Gum · Nutritional composition

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4.1 Introduction

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub], also known as *guar*, is a self-pollinated crop belonging to the Leguminosae family. It is an important legume grown mostly under resource-constrained conditions in the Indian subcontinent. Its deep-rooting growth habit aids in drought resistance and it also tolerates high temperatures (Kumar and Rodge 2012). Global production of cluster bean is about three million mt. India accounts for >2.5 million mt (USDA 2014); Pakistan follows with production of 250,000 mt. The shares of India, Pakistan and other countries in global production is depicted in Fig. 4.1. Other cluster-bean producing countries include the USA, Italy, Morocco, Germany and Spain (Punia et al. 2009). The USA, Norway, Russia, China and Germany are the leading cluster bean gum importing countries from India (Table 4.1).

Cluster bean is mostly grown for gum purposes in the northwestern parts of India, whereas in other locations it is mostly grown for vegetable use (Rai and Dharmatti 2013). The agroclimatic conditions of the Indian subcontinent (arid regions) offer the most suitable environments for its cultivation (Pathak and Roy 2015).

Cluster bean is used as a vegetable, cattle feed/fodder and green manure, being a good source of nutrition (both for humans and animals). It is also a cheap source of protein and other nutrients (Bhatt et al. 2017). Its seed consists of three important parts: husk or hull (14–17%), endosperm (35–42%) and germ or embryo (43–47%). The seed of cluster bean is a source of *cluster bean gum*, classified as a nontoxic and eco-friendly agrochemical. The gum (a galactomannan) is a naturally-occurring hydrocolloid present in the endosperm of seed (also known as *guaran*; Bhatt et al. 2017). It is a high molecular weight carbohydrate, yellowish-white in color, odorless and possesses different viscosities/granulometries (Chudzikowski 1971; Rodge et al. 2012). The gum is produced primarily from ground endosperm, after dehusking the seed (Sabahelkheir et al. 2012). Cluster-bean gum with its diverse industrial applications has emerged as a high foreign exchange earning potential crop commodity. Several varieties of cluster bean are available for different purposes: gum, vegetable, forage, fodder and cover crop (CSIR 1997). High temperatures and

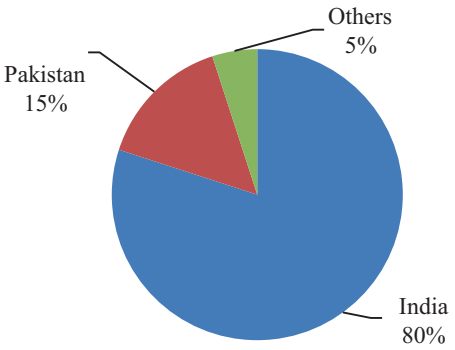

Fig. 4.1 Contribution of India to global cluster-bean production

Table 4.1 Global export of cluster bean gum from India

Country	2015–2016		2016–2017		2017–2018	
	Quantity (1000 mt)	Value (1000 USD)	Quantity (1000 mt)	Value (1000 USD)	Quantity (1000 mt)	Value (1000 USD)
United States	132,008	257.0	178,001	236.05	214,696	347.84
Norway	11,783	6.17	59,260	33.09	77,755	43.23
Russia	14,599	26.03	21,187	27.80	24,475	40.57
China	33,178	45.59	29,792	31.05	27,447	38.63
Germany	19,128	29.63	21,240	24.20	21,807	31.78
Canada	9844	18.25	8271	12.37	10,983	17.20
Netherland	2382	3.44	4716	7.04	24,998	16.63
Argentina	4372	9.08	3940	5.92	8449	13.04
United Kingdom	5814	6.95	17,164	15.66	15,796	12.88
Italy	13,688	11.61	8882	7.68	9280	9.21
Japan	4639	6.44	3856	5.73	33,367	6.78
Australia	1984	3.69	2655	3.60	3719	5.62
Brazil	2907	6.48	3120	4.76	2923	5.25
Indonesia	2343	3.47	2367	2.31	3230	4.0
Oman	963	1.14	3159	3.0	2725	3.89

Source: Agri Exchange, Agricultural and Processed Food Products Export Development Authority (APEDA), India http://agriexchange.apeda.gov.in/indexp/Product_description_32headChart.aspx?gcode=0502

relative humidity affect the quality of seed and may lead to complete loss of viability (Doijode 1989). A significant reduction in seed germination rate has been reported after 6-month seed storage (Kalavathi and Ramamoorthy 1992). Wide morphological and agronomic variability has been observed in the germplasm with respect to branching patterns, shape, size and texture of pods, size and color of seed and leaf pubescence (Dabas et al. 1995). Cluster bean with its much needed diversity for different uses is an ideal crop suitable for consumption (humans and animals) and for industrial purposes. We present here the present status, breeding, production and other aspects of this important arid land legume.

4.2 Origin and Botany

The genus *Cyamopsis* belongs to the Leguminosae family and consists of four species: *C. dentata* (N.E.Br.) Torre, *C. senegalensis* Guill. & Perr., *C. serrata* Schinz and *C. tetragonoloba* (Chevalier 1939; Dwivedi and Bhatnagar 2002). Gillett (1958) considered *Cyamopsis* a separate genus with Africa as its probable center of origin and India as the center of its variability (Vavilov 1951). The origin of cluster bean is explained by a trans-domestication process (Hymowitz 1972). The cultivated

species (*C. tetragonoloba)* was proposed to have developed from the drought-tolerant wild African species *C. senegalensis* (Mudgil et al. 2014).

Cluster bean is an annual, about 50–150 cm tall, bushy and possessing a deep tap root system with well-developed lateral roots and rhizobium nodules suitable to the climatic conditions of arid/semiarid regions (Undersander et al. 2006). Erect, branched or plants with basal branching are observed. The erect varieties have no branching or may have 1–2 branches (Fig. 4.2). The varieties with basal branching characteristics have 3 or more branches which are present at the base of the plant, while branched varieties have 4–10 branches along with the main stem. Unbranched cultivars of cluster bean have only a main stem with heavy bearing of pod clusters. The leaves are of medium size, pubescent or glabrous arranged in an alternating trifoliate pattern. It produces long purplish pink or white flowers (Fig. 4.3).

Cluster bean is cleistogamous (nonopening flowers) in nature and is a completely self-fertile and self-pollinated crop. However, 0.3–7.9% outcrossing has been reported by various researchers (Ahlawat et al. 2012; Chaudhary and Singh 1986; Saini et al. 1981). The inflorescence is a raceme and its length varies in different cultivars. Menon (1973) observed 40–60 flowers in the branched type and 50–70 in the erect type with sparsely-branched inflorescences. Flower coloration changes from white to deep blue in the bud to petal drop stage. Mostly, flower color is purplish to pink. However, white flowers are also observed in some cultivars (Fig. 4.3). A mature bud is creamy white and changes to light pink or white. Petals develop a pink color just prior to opening. The pods are flattened and borne in clusters, hence the common name (Singh et al. 2009). The pods are beaked, containing 5–12 seeds and are 2.5–13 cm long. The seeds are compressed, square in shape and grayish in color. The crop has an indeterminate growth habit and the pods at the apex of the plant remain green resulting in poor quality seeds at harvest.

Fig. 4.2 The nonbranching, single stem plant type with pod clusters

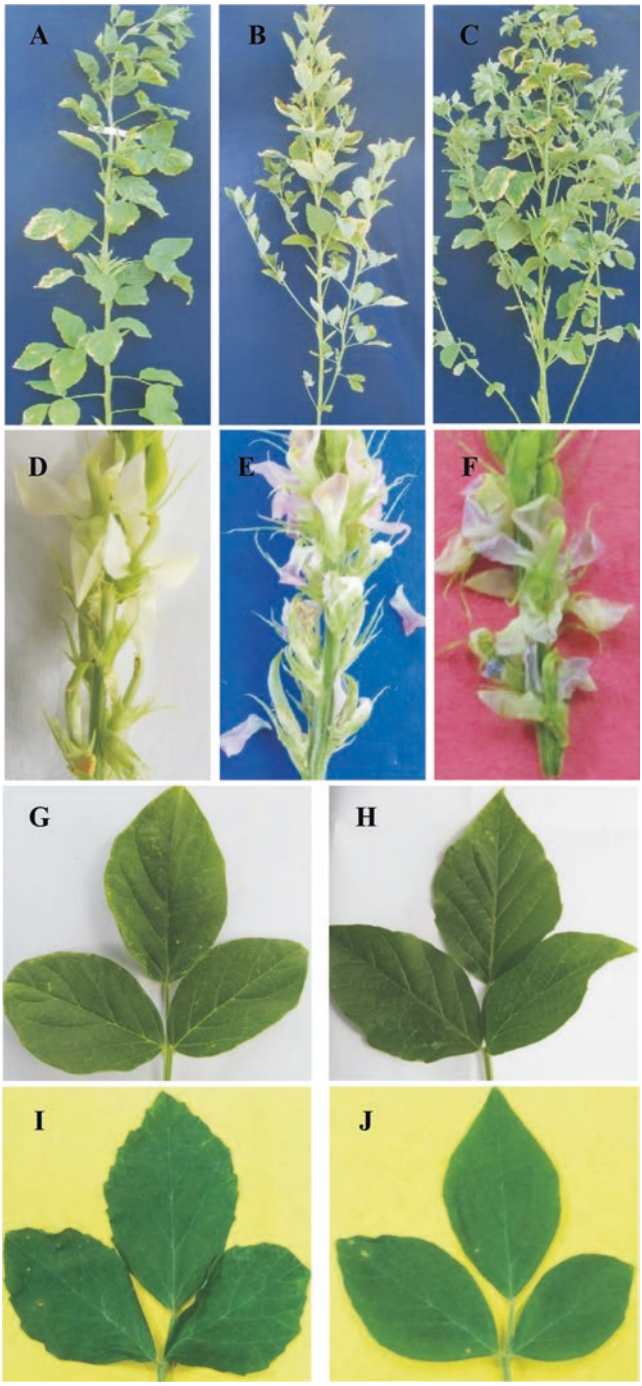

Fig. 4.3 Trait variation in cluster bean. Branching: (**a**) Unbranched; (**b**) Sparse branching; (**c**) Heavy branching. Flower color: (**d**) White; (**e**) Pink; (**f**) Purple. Leaf shape: (**g**) Ovate; (**h**) Deltoid. Leaf margin: (**i**) Serrated; (**j**) Smooth. (Source: Adapted from Bhatt et al. 2016)

4.3 Diseases and Pests

Cluster bean is grown during the rainy season; usually, common diseases of the leguminous crops are observed, but most predominant are bacterial blight, alternaria leaf spot and powdery mildew. Chemical control of these diseases is available, but sowing of disease-resistant varieties is the best option for control. Various germplasm and improved lines with disease-resistance attributes have been evaluated and identified (Gandhi et al. 1978; Gupta 1997). Various insect pests feed and grow on the leaves and pods of cluster bean (Butani and Jotwani 1984). Although the crop does not have any specific insect pests, it can be infested by pests such as aphids, blossom thrips, midges, whitefly, stink bugs, pea leaf miner and weevils (Butani 1980). Sucking pests like jassids and thrips have also been reported (Butani 1979). Whitefly is one of the major sucking pests of this crop (Patel et al. 2011).

4.4 Nutrition

The nutritional composition of cluster bean seed varies significantly as given in Table 4.2. The seed moisture, oil and crude fiber content was 7.3%, 2.3% and 9.3%, respectively (Ahmed et al. 2006). Cluster bean is mainly used for gum production, but variability in protein and fat may be of interest to plant breeders as cluster-bean meal is also an important animal feed. Cluster-bean meal, a by-product of the gum industry, consists of the outer seed coat and germ portion and possesses about 35–47.5% crude protein which is 1½ times higher than other leguminous seeds. It is used as feed for livestock, poultry and farmed fish. The ash content and mineral concentrations found in the seeds of cluster bean are comparatively higher among other pulses (Pathak 2015). The green fodder obtained from cluster bean is a good source of nutrition having 16% crude protein, 46% total digestible protein (TDN) and 60% digestive dry matter.

Table 4.2 Nutritional value of cluster bean seed

S. No.	Nutrient	Quantity
1	Fat	1.8–5.2%
2	Gum content	23.9–34.2%
3	Crude protein	28.3–35%
4	Crude fiber	4.1–8%
5	Carbohydrate	38.8–59.1%
6	Ash	3.5–6%
7	Polyphenols	25 mg/100 g
8	Phytic acid	540 mg/100 g

Source: Ahmed et al. (2006); Pathak et al. (2011a, b)

Also reported in cluster bean seed are several toxic compounds like gallotannins, gallic acid and its derivatives, kaempferol-3-glucoside, kaempferol-3-rutinoside, kaempferol-7-glucoside-3-glycoside, ellagic acid, caffeic acid and chlorogenic acid (Kaushal and Bhatia 1982).

4.5 Uses

Cluster bean is a multipurpose crop; its green pods are used as a vegetable, grain as a pulse and green plants as fodder/green manure (Fig. 4.4). It adds organic carbon to the soil by shedding its leaves gradually up until maturity thereby rejuvenating soil health.

The cluster bean seed is composed of three parts: (1) hull which constitutes about 15–17% by volume, forming the outer seed coat; (2) germ cell/embryo which is the inner soft part, representing about 35–42% by volume and (3) endosperm which represents the largest volume (43–47%) of the seed and is the source of cluster-bean splits and gum powder (Fig. 4.5) (Bhatt et al. 2017). The gum obtained from the seed is one of the best ingredients for thickening, emulsifying and as a stabilizing agent. It is stable over a wide range of pH, thereby improving the flowability and pumpability of fluids and acts as a superior reducing agent (APEDA 1999). Due to these important attributes, it has been used as the main ingredient in several products/processes in different manufacturing units (Table 4.3). Its gum has the ability to suspend solids, bind water and control the viscosity of aqueous solutions; therefore, it is used as an ingredient in different industries including paper, textile, oil drilling, mining, explosives and ore floatation. It is used in the pharmaceutical industry to stabilize liquid medicines, act as binder and disintegrating agent in tablet preparations.

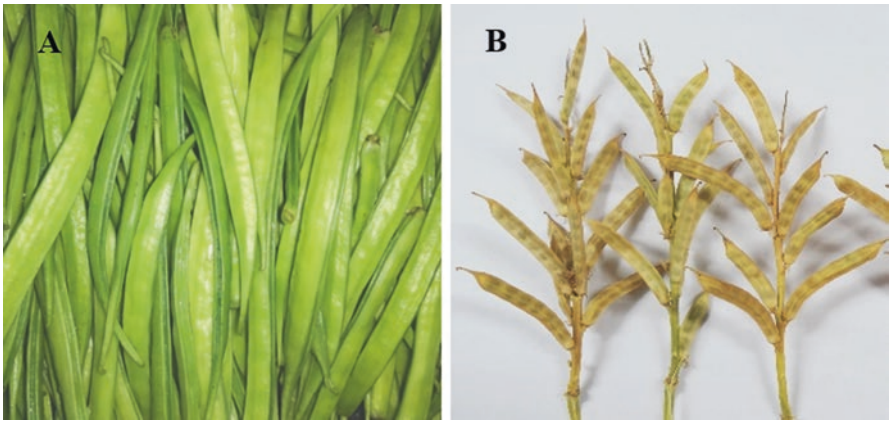

Fig. 4.4 Fresh cluster bean pods used as vegetable (**a**) and dried pods (**b**) for gum extraction purposes

Fig. 4.5 The cluster bean: (**a**) seed, (**b**) splits, (**c**) powdered form of gum

Table 4.3 Industrial uses of cluster bean

Serial number	Industry	Uses	Functions
Food Applications			
1	Bakery	Bread, cakes, pastry	Better moisture retention, increased shelf life and dough improvement
2	Dairy products	Yoghurts, desserts	Texture retention after sterilization
3	Canned foods	Pet foods, meat, baby foods	Acid resistant thickening and suspending agent
4	Animal feed	Calf milk replacer, veterinary foods	Suspending and granulating agent
5	Frozen food	Ice creams, frozen cakes	Water retention, stabilizer, and ice crystal inhibition
6	Instant mixes	Sauces, desserts, beverages	Dispersible, thickening and texturizing agent
Pharmaceutical Applications			
7	Pharmaceutical	Laxative, slimming aids	Bulking agent and appetite depressant
		Gastric acidity	Synergistic activity with bismuth salt
		Diabetic treatment	Reduces glucose loss through urine
		Cholesterol treatment	Reducing aid
		Vitamin formation and preparation	Water soluble suspension
Cosmetics			
8	Cosmetics	Shampoos and conditioners	Detergent compatible thickener and protective colloid film forming agent
		Tablets	Disintegrating and granulating agent
		Ointment	Thickening agent
		Lotions	Lubricating and suspending agent

(continued)

Table 4.3 (continued)

Serial number	Industry	Uses	Functions
Industrial Applications			
9	Textile printing	Cotton, silk, wool sizing and carpet printing	Reduces wrap breakage, reduces dusting film forming thickening for dye
10	Paper	Photographic, wrapping, craft, filter paper	Increases strength, decreases porosity and pulp hydration
11	Mining	Ore concentration and filtration	Flocculating and settling agent
12	Explosive	Stick explosive and blasting slurries	Waterproofing and gelling agent
13	Oil well drilling	Drying fluids and hydraulic fracturing	Water loss control, viscosity/ suspension/turbulence and friction reduction
14	Water treatment	Industrial and drinking water	Coagulant aid
15	Photography	Emulsions and gelatin solutions	Gelling and hardening agent
16	Ceramic	Enamels and electroceramics	Fixing, binding and thickening agent
17	Synthetic resins	Polymerization, suspension, and collagen dispersion	Thickening and binding agent

Source: Adapted from USDA (2014)

Cluster-bean gum is used in food dressings, sauce preparations and semi-liquid products. It is mixed with creams and lipstick/lip balms to produce smoothness and consistency in the products. Since it is a cost-effective hydrating agent, emulsifier and with good thickening properties, it is also utilized in paints, photography, explosives, water treatment, firefighting, ceramics, synthetic resin, wallpaper, battery electrolytes and printing ink. It is widely used in oil and gas well drilling as a shale inhibitor, for lubrication and cooling of drill bits and as a solids carrier.

The medicinal uses of cluster bean include the use of leaves as treatment for asthma, night blindness and its ash to treat skin diseases in animals. Seeds are used as a laxative, whereas boiled seeds are used to treat plague, inflammation, sprains (Khare 2004) and arthritis (Katewa et al. 2004). Its gum binds with water in the digestive tract to form a gel; the gel lowers the absorption of cholesterol and helps in the regulation of the digestive tract. It is effective in treating a number of diseases like irritable bowel syndrome, Crohn's disease, diabetes and colitis. Gum granule, tablet and capsule formulations are available.

4.6 Species Description

The genus *Cyamopsis* is divided in to four species: *C. tetragonoloba*, *C. senegalensis, C. serrata* and *C. dentata* (Gillette 1958). *Cyamopsis tetragonoloba* is cultivated throughout the world and is considered a domesticated species, while the other three are wild species. An identical number of chromosomes (2n = 14) and morphological similarity has been observed among *C. senegalensis*, *C. serrata* and *C. tetragonoloba* by karyotypic studies (Arora et al. 1985). *Cyamopsis serrata* has a longer chromosome complement as compared to *C. senegalensis* and *C. tetragonoloba*, indicating its primitive status in relation other *Cyamopsis* species. A description of the three wild species is given below.

4.6.1 Cyamopsis senegalensis

Cyamopsis senegalensis is slow-growing annual herb reaching a height of 0.5 m and covered with appressed biramous hairs (Menon 1973). The hair is not found on the upper surface of leaflets and corolla. Its leaves are narrow, pentafoliolate, persistent, linear and up to 6 mm in length. The pod is erect, slightly curved, narrowly oblongate and 4–6 cm in length with 7–9 seeds which are small, gray/white and minutely tuberculate; pod shattering occurs. The hundred-seed weight is variable, 1.0–1.4 g. *Cyamopsis senegalensis* is considered the ancestral form of the cultivated cluster bean with comparable gum and viscosity characteristics (Strickland and Ford 1984). Allozymes, restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) profiling have revealed that *C. senegalensis* is more closely related to *C. serrata* than to *C. tetragonoloba* (Hiremath et al. 1996).

4.6.2 Cyamopsis serrata

Cyamopsis serrata is also a slow growing annual species with narrow trifoliolate leaves and pink-colored seeds It is an early maturing (40–50 days) wild species possessing various agronomic and economically-important characteristics such as drought resistance (Menon 1973), photo-and thermal-insensitivity (Ahlawat et al. 2013a) and disease resistance (Orellana 1966). The seeds are small and its 100-seed weight is 1.0–1.4 g. Pod shattering is observed in *C. serrata* also. Weixin et al. (2009) found that *C. serrata* is more closely related to *C. senegalensis* than *C. tetragonoloba.* Sandhu (1988) demonstrated that *C. serrata* had 7 pairs of chromosomes and that the chromosome complement was longer as compared to the other species. In view of the longer chromosomes, it is assumed that *C. serrata* may represent a comparatively primitive status relative to other *Cyamopsis* species.

4.6.3 Cyamopsis dentata

Cyamopsis dentata is an erect branching annual herb, 30–35 cm tall. It has oppressed biramous (equally or unequally branched) hairs, with entire or dentate leaflets bearing mauve-purple flowers, and glabrous and erect pods. This species occurs in the same habitats as *C. senegalensis* and *C. serrata* (Pathak 2015) and is considered to be an intermediate species between the two.

4.7 Cultivation and Limitations

Clusterbean growth requires 90–110 days from sowing to harvesting; however, the cycle may vary from 60–90 days for determinant varieties to 120–150 days for indeterminate varieties (NRAA 2014). It is generally sown in the first to second week of July after rainfall, or up to August depending on rainfall, and harvested in October–November. The crop has also been cultivated during the summer season and its average grain yield is about three times as large, compared to the rainy season yield, suggesting that cluster bean can be successfully grown in the summer season with 4–5 irrigations (Anonymous 2013). The sowing time of the crop plays a crucial role in the growth and subsequently in the seed yield. In general, the optimum time for the sowing of crop during rainy season is the first week of July to get a higher seed yield. The summer crop may be sown during the month of March in North India and February to October in South India. Satyavathi et al. (2014) evaluated cluster bean varieties during the summer season and reported significant differences in their performances for growth, biomass production, seed yield and tolerance to pest and diseases.

Traditionally cluster bean is sown by manual broadcasting, followed by one ploughing for proper seed incorporation into the soil. But this method creates difficulty during hoeing, weeding and removing excessive water from the field. Line sowing with adequate row-to-row and plant-to-plant spacing is a better practice for proper management and productivity of the crop. The plant-to-plant and row-to-row spacing varies for different regions due to diverse rainfall intensities. A greater number of plants and higher number of pods per plant have been observed with inter-row spacing of 30 cm (Bhadoria and Kushwaha 1995). Generally, closer spacing is for fodder production while broader spacing is used for vegetable and seed production purposes. Row-to-row and plant-to-plant spacing of 45 cm and 15 cm, respectively, gives optimum seed yield.

Rhizobial inoculation of seeds is essential prior to sowing to maintain the population of bacteria in the soil. The inoculation with rhizobia controls the mortality of early seedlings and development of diseases at later stages. It does not require much nitrogen, but a modest dose of nitrogen helps to stimulate plant growth in its early stage. Studies suggest that with the application of 20 kg/ha of nitrogen as compared to no nitrogen, the crop gives higher grain/straw yield, improves water-use efficiency,

gum content and net return (Yadav et al. 1990, 1991). Cluster bean is highly sensitive to waterlogging; therefore, proper drainage is essential. Because it is a rainy-season crop, it has to compete with a large number of weeds for moisture, nutrients and space, which affects crop yield (Bhadoria et al. 1996). Therefore, weeding and hoeing (preferably within 25–30 days after sowing) is necessary to keep the field free of weeds. It has been observed that the seed yield can be increased by 61–68% by weed control alone (Yadav et al. 1993). Cluster bean varieties sown for seeds generally mature within 90–100 days. However, the varieties sown in lighter soil reach maturity 10–15 days prior to those in other soil types. Harvesting is generally carried out during morning hours to avoid seed shedding. The fodder crop is harvested at 50% flowering stage to provide good-quality fodder. Cluster bean is considered as the most preferred crop under crop rotation, especially under arid conditions. Cluster bean-wheat crop rotation is more prevalent in parts of north-western India. Similarly, higher grain yield of pearl millet was recorded when it was grown after cluster bean, as compared to continuous cropping of pearl millet in a particular field (Saxena et al. 1997).

4.8 Genetic Diversity Resources and Conservation

Progress in agricultural productivity is largely based on the development of improved cultivars with higher yield and better adaptability. The desired plant type can be obtained by exploiting the genetic diversity available among the different species of *Cyamopsis.* Old varieties or cultivars with low yield and productivity should be replaced with high-yielding, disease/pest-tolerant and nutritionally-enriched varieties. Cluster bean accessions including those from the two wild species *C. serrata* and *C. senegalensis* have been collected and 4313 accessions have been conserved (at −18 °C) at the national gene bank, maintained by National Bureau of Plant Genetics Resources (NBPGR), New Delhi, India. The promising resistance lines against bacterial leaf blight (GAUG-9406, GG-1, RGC-1027), *Alternaria* leaf blight (GAUG-9406, GAUG-9005, GG-1, GAUG-9003) and root rot (GG-1, HGS-844, GAUG-9406) have been developed by evaluating more than 375 accessions (Kumar 2008). Some of the promising lines have been released as varieties for seed (Sona, Suvidha, IC-09229/P3, Naveen, PLG-85, RGC-471) and vegetable production (Pusa Mausmi, Pusa Sadabahar, Pusa Navbahar, IC-11388, PLG-850, Sharad Bahar) Pathak (2015).

4.8.1 Genetic Diversity Resources

Knowledge of genetic divergence among varieties has significance in the development of breeding material/cultivars with higher yield and desirable quality traits (Pathak et al. 2011a; Singh et al. 2014). Wide variability of different plant types viz.,

branched or unbranched plant types, hairy or smooth stems, straight or sickle-shaped pods, pubescent or glabrous leaves, determinate or indeterminate growth habit, regular or irregular pod-bearing habit have been reported in cluster-bean germplasm (Saini et al. 1981). Additionally, the broad range of variability for different morphological and biochemical parameters of cluster bean viz., plant height (31.8–42.8 cm), number of primary branches (7.7–13.1), number of secondary branches (10.8–29.8), number of pods per plant (21.1–44.9), number of seeds per pod (6.9–9.4), 100-seed weight (2.57–3.06 g), seed yield per plant (5.5–11 g) and days to 50% flowering (30.3–35.8), endosperm (30.4–46.3%), gum content (23.5–33.5%), crude fiber (4.1–8.0%), fat content (1.8–5.2%), crude protein (28.3–35%), ash content (3.5–6%) and carbohydrate content (38.8–59.1%) have also been reported (Ahmed et al. 2006; Joshi 2002; Jukanti et al. 2015; Pathak et al. 2011a, b; Rodge 2008). Furthermore, Mishra et al. (2009) reported ranges for different traits including days to 50% flowering (25–76), days to 50% maturity (66–128), branches per plant (0–29), clusters/plant (2–86), pod length (1.6–17 cm) and seeds per plant (1.2–71 g).

The genotypic coefficient of variation (GCV) together with heritability estimates, are considered a reliable indication of improvement of a desired trait (Burton and De Vane 1953). Moderate to high estimates of GCV for seed yield and yield-attributing traits have been reported in cluster bean (Arora and Lodhi 1995; Jukanti et al. 2015; Kapoor and Bajaj 2014; Pathak et al. 2011a). Although seed yield depends on various traits, high values of GCV indicate that there are sufficient possibilities for direct selection to improve the trait. Higher variability estimates for different traits indicate a wide scope for selection and for its improvement in cluster bean (Arora and Gupta 1981). Higher GCV estimates have been reported for important characters like clusters per plant, pods per plant, seed yield per plant, and biological yield per plant (Chaudhary et al. 2003).

Heritability coupled with genetic advance is more advantageous during selection of the best genotype, than by heritability alone (Singh et al. 2010). The characters showing higher heritability and genetic advance are controlled by an additive gene effect and may be improved on the basis of phenotypic performance (Shekhawat and Singhania 2005). It has been observed that both the additive and non-additive genes have significant roles in the expression of many traits in cluster bean. Several traits of cluster bean have higher estimations for heritability and genetic advance, indicating that the seed yield and its components are governed by non-additive gene action (Chaudhary et al. 1991; Lokesh and Shiv Shankar 1990; Pathak et al. 2011b). Some traits such as days to flower initiation, plant height, number of branches per plant, number of pods per plant and number of seeds per pod are governed by additive gene action (Arora et al. 1993, 1999; Hooda et al. 1991; Pathak et al. 2011b). High heritability was found for plant height, clusters per main branch/primary branch and pods per cluster (Jukanti et al. 2015). Anandhi and Oommen (2007) and Weixin et al. (2009) have reported moderate to high heritability estimates for several traits among cluster bean varieties. Furthermore, it was suggested that selection for seed-gum content could be successful, but not as easily achieved as other characters such as pod length or seed weight (Stafford and Barker 1989). High heritability

coupled with high genetic advance for plant height, pod yield per plant, pods per plant and days to 50% flowering; while high heritability and low genetic advance for pods per cluster and seeds per pod were recorded by Rai et al. (2012). Similarly, high heritability coupled with high genetic advance estimates for stem girth, pod length, green fodder yield and dry matter yield have also been recorded (Kapoor and Bajaj 2014).

4.8.2 Genetic Resources Conservation

The genetic diversity of a cultivated crop species and its wild relatives together represent its genetic resources (Ford-Lloyd 2001). Genetic resources are the essential tools for crop improvement; their conservation and utilization are a basic requirement in any crop breeding program. Efforts to collect, evaluate, document and conserve cluster-bean germplasm were initiated in India in the 1950s (Dabas 1993). Initial efforts were concentrated in the western and northwestern parts of the country. Later, the program was intensified under the PL-480 scheme of Collection and Isolation of Superior Genotypes for Gum. These efforts up to the 1990s resulted in collection and conservation of 4878 accessions. Furthermore, exotic collections were introduced from the USA. Additionally, state agricultural universities located at Hisar, Haryana; Sardar Krushinagar, Gujarat; Durgapura, Rajasthan and Central Arid Zone Research Institute, Jodhpur, made concerted efforts to strengthen their germplasm collections. More exploration and collection efforts are required in other parts of India (central and northern), along with regions in Pakistan and Africa. Based on agroclimatic and floristic regions in India, the Indus plains are considered to exhibit uniqueness and richness in cluster bean diversity (Arora 1988; Chatterjee 1939; Murthy and Pandey 1978). Although collection and evaluation are important, proper conservation of the crop diversity is fundamental to sustainable development and food security (Sivaraj et al. 2013). Biotechnological techniques are being used in germplasm management for identification of accessions, core collection diversity estimation, to detect duplicates, to confirm true hybridization and to evaluate taxonomical status.

4.8.2.1 Ex Situ Conservation

The two major plant genetic resource conservation methods employed are ex situ and in situ. Ex situ conservation refers to conserving the genetic material outside its natural habitat, and is performed in facilities that support either storage or maintenance under suitable conditions to maintain viability and genetic composition (Sivaraj et al. 2013). Ex situ conservation helps in protecting and providing the necessary quantity of germplasm for research (Singh et al. 2004). The different ex-situ conservation approaches include: (1) plant conservation—botanical gardens, field gene banks and arboreta, (2) seed conservation—low temperature cold storage

and cryopreservation in liquid nitrogen at −150 to −196 °C, (3) in vitro conservation—storage of cells, organs, tissue under aseptic conditions, (4) DNA conservation—in the form of genomic library (whole genome) or DNA library (DNA sequence) with appropriate conservation methods. Ex-situ conservation of cluster bean genetic resources in India is being undertaken by various state agricultural universities, the All India Coordinated Research Project on Arid Legumes and the Indian Council of Agricultural Research (ICAR) institutes located across the country. These centers are designated as National Active Germplasm Sites with the responsibility of maintenance, evaluation, supply and long-term storage (−20 °C) of germplasm.

4.8.2.2 In Situ Conservation

The in situ conservation methods involve the conservation of crop genetic resources within their natural habitats (Sivaraj et al. 2013). Mostly, in situ conservation is employed for endangered forestry species, species belonging to complex ecosystem and wild relatives. This method includes two approaches: (1) ecosystem approach—biosphere reserves and (2) habitat approach—national parks, gene sanctuaries and sacred groves. Habitat maintenance is an integral part of in situ conservation. Therefore, to a certain extent in situ methods could directly deal with causes responsible for environmental degradation and help to limit their impact.

4.8.3 Inheritance Studies

Inheritance studies in cluster bean have revealed that foliage color, branching and pollen fertility are controlled by a single gene, while pod length is controlled by several genes (Dabas 1975; Saharan et al. 2004). The dominance of dark-green foliage over light-green, pollen fertility over sterility and unbranching over branching was observed. The dominance of serrated leaf margin over a smooth margin, pubescent leaf surface over the glabrous surface (Singh et al. 1990), hairy type over nonhairy, branched over unbranched, purple flower over white flower, broad leaf over narrow leaf, curved pod over noncurved pod, plucked leaf surface over nonplucked leaf surface (Saharan et al. 2004), have been observed in cluster bean. Furthermore, a single dominant gene was found to be responsible for branching pattern and leaf serration (Saharan et al. 2004).

In order to develop high gum yielding cluster-bean genotypes, it is essential to know the type of gene effects controlling the expression of gum content in the seed. The inheritance of seed-gum content is quite complex and both additive/non-additive gene action are involved in its expression (Dabas 1975). Additionally, seed gum content has been reported to be controlled by additive, dominant and epistatic effects with further modification by the environment. Breeding efforts for

improvement of seed-gum content in cluster bean have been mostly hindered due to the influence of additive and non-additive gene action and its negative association with seed weight, but a positive relation with seed yield (Jhorar et al. 1989; Pathak et al. 2011a; Weixin et al. 2009).

4.9 Genetic Improvement Approaches

Cluster bean is a legume crop with resilience to conditions of heat and drought, in addition to being nutritionally rich in nature. More significantly, it has emerged as an important industrial crop with good potential for earning foreign exchange. This crop is an important alternative for farmers in arid and semiarid regions of India and the world. Therefore, high yield, stress tolerance and high seed gum content, combined, represent the major breeding objectives of cluster bean improvement across the globe. A narrow genetic base, limited breeding efforts and limited use of advanced molecular tools are major bottlenecks to cultivar improvement. But, the situation has changed significantly in recent years. Several new molecular techniques are being used to study and improve this crop. Genomic and tissue culture-based tools have been developed which can aid in developing improved cultivars. The following are the genetic improvement techniques that are presently being used in cluster bean improvement programs.

4.9.1 Hybridization

Genetic variability in any crop can be developed through interbreeding of different species or genetically divergent individuals from the same species (Harrison and Larson 2014). Generally, hybrid progeny developed through hybridization is observed to perform better with increased yields. Cluster bean has small cleistogamous flowers and is considered a highly self-pollinated crop with low levels of outcrossing (0.5–7.9%) (Saini et al. 1981). Therefore, hybridization in cluster bean is very difficult and painstaking. Different varietal improvement methods including pedigree, bulk pedigree and backcross are commonly used in self-pollinated crop species for traits which are seed related, disease resistance and maturity (Knauft and Ozias-Akins 1995). Although these methods have helped in developing varieties with better yield, there is still a need for further development of new and better high-yielding varieties and hybrids, keeping in mind the challenges of ever-increasing human population and drastic climate change. Some characters like earliness can be transferred from wild species (*Cymopsis serrata*, *C. senegalensis*) to cultivated cluster bean by hybridization. Additionally, landraces and germplasm accessions of cluster bean can be a very good source of variation for different agronomic important traits. FS 277 and HG 884 are two cluster bean genotypes with high yield, gum,

protein and proline content which have been successfully utilized in hybridization programs (Buttar et al. 2008; Jitender et al. 2014).

Interspecific hybridization in cluster bean is limited due to stigmatic incompatibility and lack of pollen germination (Sandhu 1988). Interspecific hybridization using both conventional and nonconventional breeding methods was attempted between *Cymopsis tetragonoloba* x *C. serrata* and *C. tetragonoloba* x *C. senegalensis* (Ahlawat et al. 2013a). Hybridization was successful only between *C. tetragonoloba* × *C. serrata.* A total of 792 crosses were made but only 83 pods were recovered for the *C. tetragonoloba* x *C. serrata* cross, amounting to 10.47% pod set. However, no pod setting was observed using the conventional breeding method in either cross. Among the different nonconventional breeding methods used, smearing the stub of female parent (*C. tetragonoloba)* with solidified pollen germination medium (PGM) prior to pollination was successful in the *C. tetragonoloba* x *C. serrata* cross only. Other methods like bud pollination or stigma/style amputation failed to overcome stigmatic incompatibility barriers. Different morphological phenological characters like flower color, pod shape, height, pod size, pods per plant, pods per cluster, seeds per pod and position of trifoliate leaves indicate the hybrid nature of the plants.

The failure of hybrid seed formation by conventional breeding methods could be due to stigma incompatibility, since smearing of the stub with PGM is effective, whereas with the stigma it is ineffective and did not support any in vivo pollen germination (Ahlawat et al. 2013a) Additionally, differences in the nutritional requirement of *Cyamopsis* species pollen, the long lag phase for pollen germination in the wild species, duration of stigma receptivity and style length of *C. tetragonoloba*, are other probable hindrances to successful interspecific hybridization. The failure of *C. tetragonoloba* x *C. senegalensis* could be due to lack of in vivo germination on the PGM smeared stub. Therefore, other techniques like tissue culture and transfer of desirable genes through recombinant DNA technology could be alternative methods of hybridization.

Heterosis and combining ability are important approaches in crop improvement for parental selection (Vasal 1998). Furthermore, the knowledge of combining ability along with gene action is essential for identifying better combiners which could be useful in exploiting heterosis and developing hybrids with improved yield ability (Nigussie and Zelleke 2001). Heterosis has been reported in cluster bean (Chaudhary et al. 1981) but a source of cytoplasmic/genetic male sterility has not been identified so far. Limited studies reported on heterosis and the combining ability of seed yield and other related traits in cluster bean (Arora et al. 1998; Saini et al. 1990). High specific combining ability (SCA) for different traits (seed yield, yield-related traits, disease resistance) has been reported in crosses involving genotypes AG 111, HFG 516, HFG 590 and Durgajay (Hooda et al. 1999). Overall, it may be concluded that although hybrids are possible in cluster bean, it could be a major challenge for crop breeders without the availability of a stable male sterility system.

4.9.2 Tissue Culture

Tissue culture techniques like micropropagation and genetic transformation could be used as supplementary approaches to conventional breeding in cluster bean improvement. The advantages of micropropagation methods include: (1) avoidance of post-fertilization barriers, (2) large-scale production of single gendered true-to-type plants (Kumar et al. 2010a, b), (3) production of plants with a low germination rate (Modi et al. 2012), (4) increase the production of secondary chemicals and (5) establishment of regeneration protocols for genetic transformation (Karupussamy 2009). Several factors (e.g. explants, medium composition, hormones, culture condition) influence the success rate of in-vitro regeneration of a plant species (Kalia et al. 2014). Researchers have attempted to establish protocols for micropropagation of cluster bean (Ahlawat et al. 2013b; Ahmad and Anis 2007; Gargi et al. 2012; Mathiyazhagan et al. 2013; Prem et al. 2003, 2005; Ramulu and Rao 1987, 1989, 1993, 1996; Sheikh et al. 2015; Verma et al. 2013).

Several protocols for establishment and enhancement of callus in cluster bean have been reported using Murashige and Skoog (MS) and Gamborg (B5) media (Ramulu and Rao 1989, 1991, 1993, 1996). Direct differentiation from cotyledonary nodes (Prem et al. 2003; Fig. 4.6) and shoot organogenesis via callus culture (Prem et al. 2005) has been reported in cluster bean. Callus induction was achieved both in cultivated and wild species (*Cyamopsis serrata* and *C. senegalensis*) using cotyledon explants (Ahlawat et al. 2013b). Multiple-shoot induction was shown with 2,4- dichlorophenoxyacetic acid (2, 4-D) and 6-benzylaminopurine (BAP) and gibberellins (Gargi et al. 2012). Meghwal et al. (2014) demonstrated the same with gibberellins, B5 media and kinetin. Plant regeneration was achieved by Verma et al. (2013) using cotyledon explant in a medium containing indole-3-butyric acid (IBA), BAP and gibberellins. Rooting followed by transfer of rooted shoot plantlets and successful hardening on cocopeat mixture was demonstrated. Somatic embryogenesis using mature embryos on MS medium containing 2 mg L^{-1} naphthaleneacetic acid (NAA), 0.5 mg L^{-1} and 3 g L^{-1} charcoal was also demonstrated by Mathiyazhagan et al. (2013).

4.9.3 Genetic Transformation

Genetic transformation studies in cluster bean were initiated by Prem (1999), using *Agrobacterium tumefaciens*, and there was observed good expression of *β-glucuronidase* (GUS) in 14-day-old explants. Stable transformants in cluster bean were produced by transforming with *β–glucuronidase* and *neomycin phosphotransferase* genes. A selection efficiency of 30% and transformation efficiency of 1.2% was shown with kanamycin sulphate (145 mg L^{-1}). Another transformation attempt in cluster bean with an α-galactosidase gene from coffee senna (*Senna occidentalis*), although successful, resulted in a 30% reduction in galactomannan content in

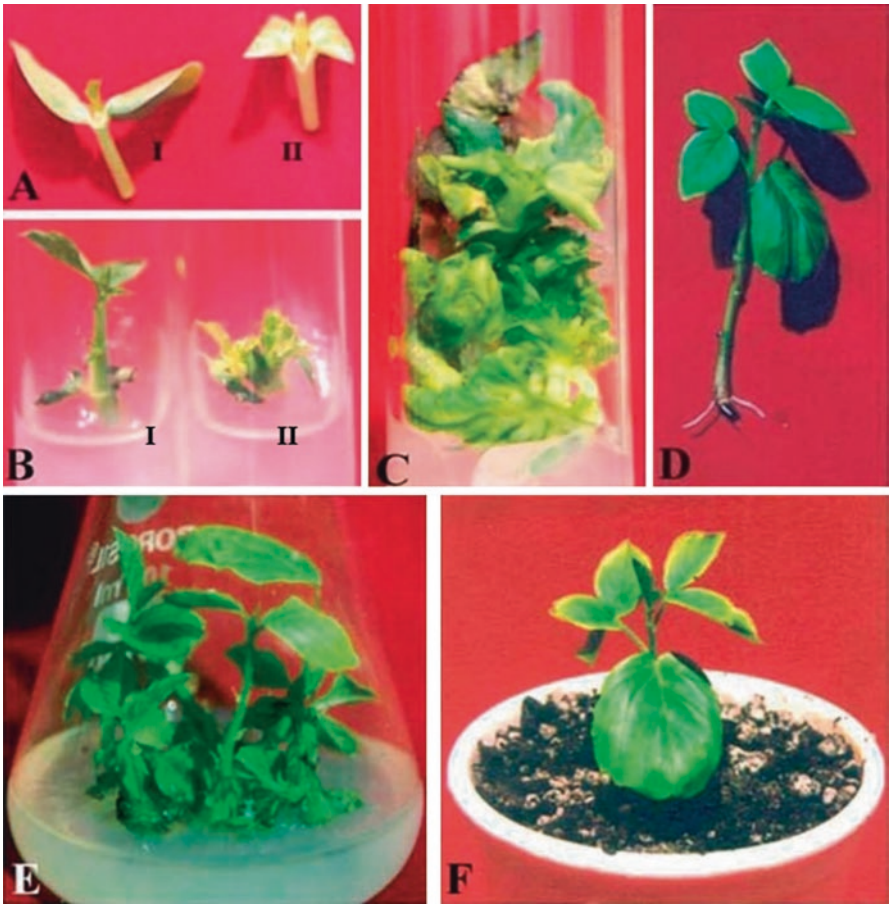

Fig. 4.6 Direct shoot-bud differentiation from cluster bean cotyledonary node explants. (**a**) Explant types: type I cotyledonary node with main shoot and type II cotyledonary node after removal of main shoot. (**b**) Type II explant showing multiple shoot initials on MS + 5.0 μM TDZ in a 4 week-old culture. (**c**) Multiple shoot buds on a cotyledonary node explant on 5.0 μM BA +0.5 μM IAA, initially exposed to 5.0 μM TDZ. (**d**) Ex vitro rooted shoot, after 15 min treatment with 300 μM IBA. (**e**) Elongated shoots on 5.0 μM BA +0.5 μM IAA. (**f**) An acclimatised plantlet in an 8-cm diameter cup. (Source: Ahmad and Anis (2007); copyright © The Journal of Horticultural Science & Biotechnology Trust, reprinted by permission of Taylor & Francis Ltd)

the transformants (Joersbo et al. 2001). Interestingly, soybean transformants expressing the *ManS* gene of cluster bean resulted in significant localization of gum in soybean seeds (Dhugga et al. 2004). An enhanced level of tissue-specific expression of a transgene is very important. The *rsu3,* promoter of sucrose synthase from rice was shown to be very strong for endosperm expression in cluster bean (Rasmussen and Donaldson 2006). Since *rsu3* is suitable for endosperm expression, this promoter could potentially be utilized to enhance the galactomannan content in cluster bean seed.

4.9.4 Mutation Breeding

Mutation breeding is a useful tool to induce genetic variability essential to crop improvement. Mutations can be induced in seed and vegetatively-propagated crops (Bhosle and Kothekar 2010). Mutagenesis involves large-scale evaluation and identification of plants with desirable traits (with no drag of undesirable traits) that could be utilized in breeding. Natural mutations are of little use to breeders as they are sudden, unexpected and rarely occur. Mutation rates have been estimated in several organisms (Kovalchuk et al. 2000). Chlorophyll deficiency and albinism are the easiest phenotypes for estimating mutation rates in plants. Mutations leading to chlorophyll deficiency occur at rates of 3.2×10^{-4} and 3.1×10^{-4} events per nuclear genome per generation in barley (*Hordeum vulgare*) and buckwheat (*Fagopyrum esculentum*), respectively. Albino phenotypes in barley were estimated to occur in about 300 different nuclear genes (Klekowski 1992). Spontaneous point mutation rates in the range of 10^{-7} to 10^{-8} per base pair and generation were reported in *Arabidopsis thaliana* based on functional reversions of a mutated gene (*uidA)* (Kovalchuk et al. 2000; Swoboda et al. 1994). Spontaneous point mutation rates of 7×10^{-9} base substitutions per site per generation were revealed upon genome sequencing of mutation accumulation lines of *Arabidopsis* (Ossowski et al. 2010).

Natural mutations in cluster bean have been reported, such as male sterility and partial male sterility (Mittal et al. 1968); and rosette-type inflorescence with reduced fertility (Stafford 1988, 1989). Although these mutants have been reported in cluster bean, their practical use in crop improvement has not been demonstrated (Arora and Pahuja 2008). In order to enhance the mutation frequency rate and utilize it in crop breeding, several physical and chemical mutagens have been identified and used successfully in crop improvement (Mullainathan et al. 2014). Various physical (X-ray and gamma rays, UV light, high energy ion beam) and chemical mutagens (ethyl methanesulfonate (MS), diethyl sulfonate (DES), sodium azide (SA), methyl methanesulfonate (MMS), nitroso guandine (NG) and nitroso methyl urea (NMU) have been utilized to induce mutations at high frequency in different crops (Pathak 2015).

Physical mutagens have been successfully utilized in generating variability that has been/or could be used in crop improvement, especially in self-pollinated crops (Tariq et al. 2008). Initially X-rays were used but later gamma rays using different sources (^{60}Co and ^{137}Cs) have gained popularity (Auerbach and Robson 1946). Gamma rays (^{60}Co as source) were the first physical mutagens successfully employed in cluster bean (Vig 1965). Gamma ray irradiated plants exhibited low fertility and a trisomics nature (Singh 1972). Gamma ray treatment resulted in reduced germination percentage, pollen fertility and seedling survival with increased dosage (10–200 kR) (Lather and Chowdhury 1972). Additionally, it resulted in various chromosomal abnormalities. On the contrary, Chaudhary et al. (1973) observed an increase in yield, gum and grain protein content using low dosage (2–20 kR) gamma ray irradiation. Chowdhury et al. (1975) reported wide variation for different characters in irradiated populations of two cluster bean varieties in M_2 generation

viz., reduced number of branches/yield per plant but, increase in peduncle length and plant height. Similarly, an increase in variability following gamma ray irradiation for different morphological, seed yield/related traits (Amrita and Jain 2003; Yadav et al. 2004) and increased frequency of chlorophyll mutants (Patil and Rane 2015) was reported.

The most interesting mutant developed by gamma ray irradiation in cluster bean was the early flowering and determinate type (Singh et al. 1981). The determinate type plants had reduced plant height, nonbranching habit, increased cluster size, early and synchronous maturity and, most importantly, the main shoot culminated either in a leaf or inflorescence. Furthermore, a 10 kR X-ray treatment of Pusa Navbahar seeds generated a true-breeding, early-flowering mutant with increased number of pods (Rao and Rao 1982). An interesting set of experiments were carried out using UV-B, UV-A and *white light* on Pusa Navbahar seedlings (Lingakumar and Kalandaivelu 1998). The UV-B treatment alone resulted in reduced pigment content, photosynthetic activity and overall plant growth. But, UV-A supplementation reversed some of the effects of UV-B by promoting the overall seedling growth and increased chlorophyll synthesis and carotenoids. But, UV-B irradiation followed by white light did not activate the UV-B damage. Furthermore, the UV-B induced reduction in quantity of photosynthetic pigments and O_2 evolution was partially reversed by UV-A + UV-B treatment (Joshi et al. 2007)

The application of a physical mutagen requires sophisticated apparatus and besides it may result in deletion of DNA and cause chromosomal aberrations with detrimental effects. However, chemical mutagens do not require specialized equipment, provide high mutation frequency and are considered more viable options compared to physical mutagens (Heslot et al. 1961). Ethyl methanesulfonate (EMS), an alkylating agent, is the most commonly used due to its ease of handling/disposal and effectiveness (Hajra 1979). Sodium azide (SA), another popular mutagen that is inexpensive, comparatively safe with no carcinogenic effects and is reported to induce high frequency point mutations with minimal or no chromosomal aberrations (Nilan et al. 1973). Cluster bean plants produced from EMS-treated seeds were found to have chlorophyll deficiency with profuse vegetative growth (Gohal et al. 1972), modified leaf shape/texture, growth habit and pod size (Swamy and Hashim 1980). A few pod mutants exhibited pleiotropic phenotypes including extensive branching, delayed flowering and change in seed color (normal violet to light gray/brown). Treating Pusa Navbahar seeds with either kitazin (200, 400, 600 ppm) or Saturn (1000, 2000, 3000 ppm) for 12 and 24 h, resulted in determinate growth habit and spreading variants (Rao et al. 1982).

Mutation induction using both physical and chemical mutagens has also been studied in cluster bean and a variety developed using both mutagens has been released (Chopra 2005). Interestingly, the combination treatment of physical (80 and 100 kR gamma rays) and chemical (aqueous solution 20.1–0.3% EMS and 0.01–0.03% N-methyl-N-nitrosourea) mutagens on the seeds of cv. Suvidha and PLG 143 produced mutants with increased number of pods, pod length and early maturity (Singh and Agarwal 1986). Additionally, these mutants were high yielding with increased gum content in the endosperm and seed. Gamma rays and sodium

azide treatment in cluster bean led to heterophylly (Badami and Bhalla 1992). Gamma rays in lower doses (up to 50 KR) and EMS in lower concentrations (< 0.2%) may be applied to obtain viable mutations in cluster bean (Dube et al. 2011; Pathak 2015; Velu et al. 2012).

Although use of different physical and chemical mutagens has provided a new perspective on hybridization of this crop, it is necessary to undertake more systematic studies to obtain desirable results. It is observed that larger levels of useful variability are produced by lower doses of various mutagens than higher doses. Limited numbers of cluster bean mutants with useful traits are available so far. Therefore, extensive experimentation involving a large number of lower doses of different mutagens, physical, chemical or in combination, to induce desirable genetic variability is essential. The variability generated could be exploited in breeding cultivars possessing high yield, early maturing, disease tolerance and high seed gum content.

4.9.5 Biotechnological Approaches

The availability of genetic diversity and its systematic evaluation is very important to successfully implement any crop improvement/breeding program. Earlier, morphological traits were used to assess the diversity and selection. But, diversity evaluation based on morphological markers is prone to environmental influences thereby sometimes making selection fruitless (Kumar et al. 2013, 2015, 2017; Pathak et al. 2011b, c). The advent of DNA-based genomic markers has been very useful in genetic diversity assessment, cultivar purity and identification, and to aid in parental selection for hybrid development. Different types of molecular markers have been employed to study the genetic diversity in cultivated varieties, genotypes and landraces of cluster bean (Boghara et al. 2015; Gresta et al. 2016; Kumar et al. 2015; Kuravadi et al. 2013, 2014; Patel et al. 2014).

4.9.5.1 Protein-Based Markers

The use of isozymes in genetic studies has been reported in several crops, weeds and wild plant species; their advantages and constraints have been widely discussed (Pérez de la Vega 1993; Soltis and Soltis 1989). According to Markert and Møller in (1959), isozymes or isoenzymes are different molecular forms of an enzyme/protein that may have the same enzymatic function. Isozymes were the first protein-based marker system used for genetic mapping in plants, and, in the past, have been extensively used as molecular markers (Weeden and Lamb 1985). These markers represent a quick and cheap system that can be used to identify low levels of genetic diversity. Isozyme markers have been used in cluster bean on a limited scale to study the genetic diversity. For the first time in cluster bean, the process of domestication was studied using the isozyme diversity in landraces, cultivars from USA

and wild varieties (Mauria 2000). Brahmi et al. (2004) reported greater inter-population diversity compared to the overall genetic diversity in cluster bean germplasm accessions through allozyme-marker studies. Although protein-based markers could be useful, due to certain constraints like fewer loci, low levels of polymorphism and inconsistency, they have been replaced by DNA-based marker systems that are more efficient and resourceful (Kumar et al. 2017).

4.9.5.2 DNA-Based Markers

DNA-based markers have been extensively utilized to study the genetic diversity and phylogeny in cluster bean. The most preferred marker system in cluster bean diversity analysis has been random amplification of polymorphic DNA (RAPD) (Ajit and Priyadarshani 2013; Kalaskar et al. 2014; Kuravadi et al. 2013; Patel et al. 2014; Pathak et al. 2011c; Punia et al. 2009; Sharma and Sharma 2013). A RAPD profile of 35 cultivars is given in Fig. 4.7. Although RAPDs have been widely used in cluster bean, inter-simple sequence repeat (ISSR) markers were reported to be more robust and efficient in revealing polymorphism and diversity (Sharma et al. 2014a, b). Kuravadi et al. (2013) studied the genetic diversity among 29 landraces and 19 commercial cultivars of cluster bean using RAPD and ISSR markers. They reported an average polymorphism of 87.63% using 13 RAPD primers, among which OPQ-09 produced the highest number of bands (12 bands). RAPD studies (used 15 primers) in 5 cluster bean cultivars (RGC-936, RGC-1002, RGC-1003, RGC-1031, RGC-1017) exhibited an average polymorphism of 66% with a range of 18.1–100% (Sharma and Sharma 2013). Furthermore, Pathak et al. (2010) studied the diversity using RAPD markers among 32 genotypes procured from the major cluster-bean-growing regions (Rajasthan, Haryana, Gujarat) in India. Polymorphism percentage expressed a range of 66.6–87.5% and OPA-16 formed the highest number of amplified products (16 products).

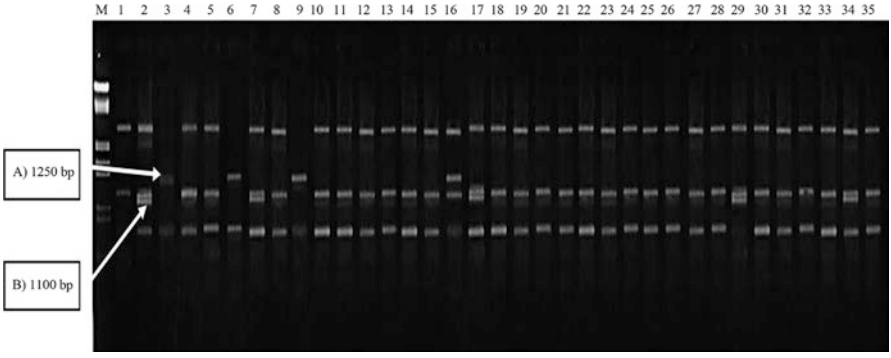

Fig. 4.7 RAPD profiling of 35 cluster-bean genotypes (lane1–35) with primer RP-3, Lane M-Lamda *Eco*R1/*Hind* III double digested. (Source: Adapted from Sharma et al. 2014a)

Sharma et al. (2014b) estimated the genetic diversity and relationships among 35 genotypes using 10 ISSR markers. A total of 105 bands were produced among which 102 were polymorphic indicating a polymorphism of 97% with a band size of 450–3500 bp. The primer efficiency was estimated using discriminatory power (Dj), which ranged from 0.44–0.99. ISSR studies using 7 markers in 48 genotypes including landraces produced 64 bands, of which 50 were polymorphic. Among the primers used, UBC-868 produced the highest number of bands (13) and the average polymorphism was 77.82% (Kuravadi et al. 2013). Molecular evaluation of genetic diversity using ISSR markers among a large set of genotypes (104) collected from different regions of India revealed a polymorphism of ~97.0% (Ansari et al. 2016).

Despite being simple to use, efficient and with no requirement of prior sequence information, both RAPD and ISSR marker systems have poor reproducibility and stability. To overcome these constraints, RAPD markers were converted to sequence-characterized amplified region [SCAR] markers (Paran and Michelmore 1993). SCAR markers are codominant, locus-specific with high reproducibility (Dhawan et al. 2013; Shah et al. 2015). SCAR markers have been developed in cluster bean from RAPD and ISSR, specific to region and genotype (RGC-1031), respectively (Sharma et al. 2014a). The variation in number and arrangement of nucleotides of the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) are considered versatile genetic markers for diversity analysis (Beltrame-Botelho et al. 2005; Powers et al. 1997) and have been successfully used in cluster bean (Pathak et al. 2011d). Pathak et al. (2011d) have identified high frequencies of single nucleotide polymorphisms in the conserved DNA sequences.

Despite being a crop of high commercial value, the availability of genomic resources and adoption of omics technologies are very limited in cluster bean. The availability of sequence-based DNA markers like SSRs is very much limited in cluster bean (Kumar et al. 2015), thereby affecting the marker-assisted breeding efforts. About 300 expressed sequence tag (EST) based SSRs (EST-SSRs) have been identified (Kumar et al. 2015; Kuravadi et al. 2014) from about 16,476 EST sequences available at the National Center for Biotechnology Information (NCBI) website (Naoumkina et al. 2007).

Genetic diversity analysis based on EST-SSRs demonstrated a narrow genetic base among the analyzed cluster bean genotypes and distributed them into three different clusters irrespective of geographical origins (Kumar et al. 2015) (Fig. 4.8) in contrast to the earlier reports (Sharma et al. 2014b). This difference in similarities could be due to the usage of different marker systems.

4.9.5.3 Transcriptomics and miRNA Studies

Transcriptomic studies are useful for large-scale discovery and characterization of functional genes and genome assembly. Additionally, RNA sequencing is considered the standard for annotating coding and non-coding genes (Adams et al. 1991; Haas et al. 2002). The RNA-Seq method presents a holistic view of the transcriptome and provides several new insights (novel transcribed regions, genic

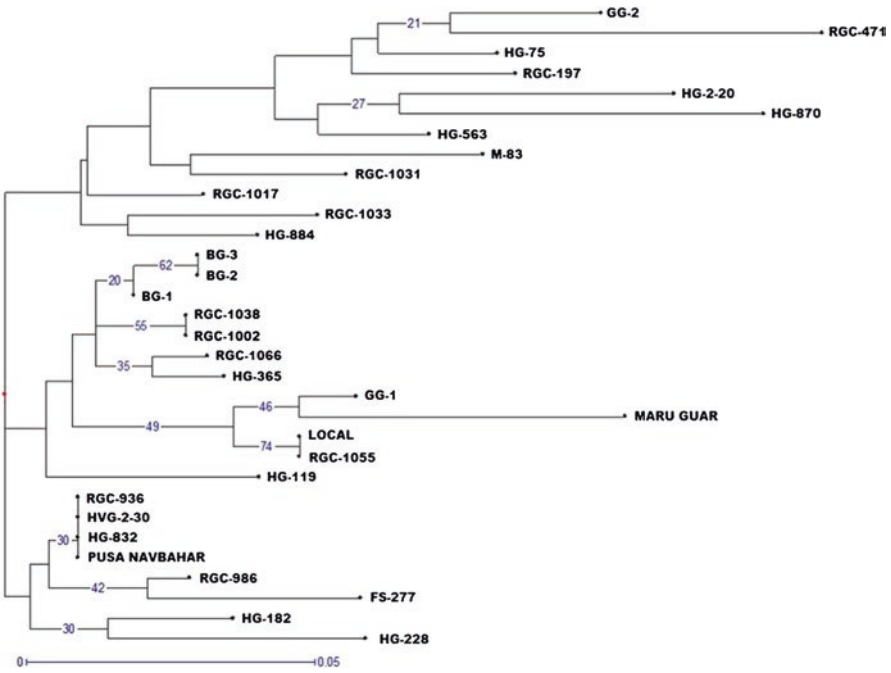

Fig. 4.8 Discrimination of 32 cluster bean genotypes using 39 EST-SSRs. (Source: Adapted from Kumar et al. 2015)

microsatellites) that could significantly aid in crop improvement (Cloonan et al. 2008; Li et al. 2010; Wang et al. 2009; Wilhelm et al. 2010). Data from a recent transcriptomic study of cluster bean leaf, shoot and flower tissue identified 127,706 transcripts and 48,007 non-redundant high-quality unigenes (Rawal et al. 2017). About 79% of identified unigenes were annotated using different databases including NCBI, KEGG, gene ontology (GO) and Swiss-Prot. Furthermore, they have identified 8687 potential SSRs with an average frequency of 1 SSR per 8.75 kb. They developed a database, Cluster geneDB, for easy retrieval of unigenes and microsatellite markers. The resources developed through this study (tissue specific genes, molecular marker) will aid in genetic improvement of cluster bean. RNA-Seq was used to study the transcriptome of cluster-bean leaf tissues of two popular varieties i.e., M-83 and RGC-1066 (Tanwar et al. 2017). This study identified a total of 62,146 non-redundant unigenes with 175,882 GO annotations. Furthermore, 11,308 unigenes were annotated and characterized into 6 clusters and 55 subclasses. A total of 5773 potential SSRs and 3594 high quality single nucleotide polymorphisms (SNPs) were reported.

Small RNA/microRNAs (miRNAs) are 20–24 nucleotides (nt) long non-coding RNAs that are universal and highly conserved (Ambros et al. 2003; Tyagi et al. 2018). They play an important role in various aspects of plant growth, development and metabolic regulation. To date, mature and precursor miRNAs are reported in

only 9 taxa of the Leguminosae: *Acacia auriculiformis, A. mangium, Arachis hypogaea, Glycine max, G. soja, Lotus japonicas, Medicago truncatula, Phaseolus vulgaris* and *Vigna unguiculata* (Kozomara and Griffiths-Jones 2014). A tissue-specific study in cluster bean variety RGC-936 was undertaken to understand the role of mi-RNAs in galactomannan biosynthesis regulation (Tyagi et al. 2018). This study identified 187 known and 171 novel miRNAs that were differentially expressed, among these 10 miRNAs were validated. ManS (mannosyl transferase/mannan synthase) and UGE (UDP-D-glucose 4-epimerase) were the 2 novel unigenes annotated and validated as targets for 3 novel miRNAs (*Ct*-miR3130, *Ct*-miR3135, *Ct*-miR3157). These findings could be useful in understanding the regulation of galactomannan biosynthesis and in future be used in breeding efforts to increase the gum content in cluster bean.

4.10 Conclusions and Prospects

Hybridization, mutation breeding and selection/evaluation of germplasm/varieties are major approaches to varietal improvement and new cultivar development. Genomics (marker-assisted selection, MAS) aided by the latest sequencing and molecular techniques can be used for identification of QTLs/genes and introduce them into adapted cultivars, could be a better approach to obtain photo-insensitive, drought tolerant, disease/pest resistant cultivars with higher yield. The crop has indeterminate growth habit and a large percentage of blackened seed is present at harvest, resulting in a reduction of seed quality. Therefore, varieties with synchronous maturity should be developed. Since interspecific crosses between wild species are successful, while crosses between wild and cultivated species are unsuccessful, the molecular approaches, including tissue culture techniques, may be exploited to overcome this fertility problem. Overall, cluster bean is an important legume with good economic value. Therefore, the development of varieties suitable for seed, vegetable and gum purpose should be the major objectives in any breeding programs.

Appendices

Appendix I: Research Institutes Related to Cluster Bean

Institution	Specialization and research activities	Website
Central Arid Zone Research Institute	Cluster bean collection, evaluation and breeding	http://www.cazri.res.in
Anand Agricultural University, Anand	Cluster bean collection, evaluation, breeding and molecular characterization	http://www.aau.in

(continued)

Institution	Specialization and research activities	Website
SKRAU, Bikaner	Cluster bean collection, evaluation and breeding	http://www.raubikaner.org
TNAU, Coimbatore	Cluster bean evaluation and breeding	http://www.tnau.ac.in
CCSHAU, Hisar	Cluster bean collection, evaluation and breeding	http:/www.hau.ac.in
PJTSAU, Hyderabad	Cluster bean evaluation and breeding	http://www.pjtsau.ac.in
ANGRAU, Gunutr	Cluster bean evaluation and breeding	https://www.angrau.ac.in
Banasthali University	Cluster bean evaluation and breeding	http://www.banasthali.org
NRCPB, New Delhi	Cluster bean evaluation and molecular characterization	http://www.nrcpb.res.in
NBPGR, New Delhi	Cluster bean collection, evaluation, and characterization	http://www.nbpgr.ernet.in
AU, Jodhpur	Cluster bean collection, evaluation, and characterization	http://www.aujodhpur.ac.in
SK Dantewada Agricultural University, Sardarkrushinagar	Cluster bean collection, evaluation and breeding	http://www.sdau.edu.in
SKNAU, Jobner	Cluster bean collection, evaluation and breeding	http://www.sknau.ac.in
RARI, Durgapura	Cluster bean collection, evaluation and breeding	http://www.raridurgapura.org

Appendix II: Cluster Bean Genetic Resources

Cultivar	Important traits	Yield (q/ha)	Cultivation location
RGC-936	Early maturing (85–90 days) branched variety suitable for rainfed and irrigated conditions. Also, resistant to multiple diseases.	8–10	India
RGC-1002	Early maturing (80–90 days) branched variety with toothed (along margins) leaves. This variety is suitable for rainfed and irrigated conditions.	10–12	Arid zones of India
RGC-1003	Branched variety with smooth leaf margins. It is suitable to rainfed conditions. Gum content in seed is ~30.0%.	10–12	India
RGC-1066	This variety is single stemmed, erect and bold seeded. Flowers are usually purplish in color. It is suitable for kharif (rainy season) and summer cultivation.	10–15	Rajasthan, India

(continued)

Cultivar	Important traits	Yield (q/ha)	Cultivation location
HG-365	It has brisk podding behavior. This variety is suitable for summer and rainfed conditions.	12–15	Madhya Pradesh and Haryana, India
HG 2–20	This variety can be grown under rainfed, irrigated or summer conditions. It is a branched and bold seeded variety.	12–15	Northern India
GC-1	Branched variety suitable for low fertile soils with good plant height (80–100 cm).	10–12	Gujarat, India
RGC-1017	Branched variety with trifoliate and toothed leaves.	12–14	India
HGS 563	Variety with brisk podding ability, pink colored flowers and high seed gum content (33%).	12–13	Haryana, India
RGM-112	Branched type with moderate resistance to bacterial leaf blight and root rot.	12–14	Rajasthan, India
RCG-1038	Branched variety with heavy podding ability and probably photo insensitive.	12–15	India
Esser	Medium to late maturing variety with good disease resistance	9–14	USA
Lewis	Medium to late maturing variety with high yielding ability	11–16	USA
Kinman	Early maturing type with resistance to bacterial leaf blight and *Alternaria* leaf spot.	7–13	USA
Sant Cruz	Sparsely branching, indeterminate and glabrous cultivar suitable for high altitudes.	20	High altitude regions in USA
BR-99	Early maturing variety, single stemmed with high yielding potential. Suitable for irrigated and non-irrigated conditions.	~20	Punjab Province, Pakistan
BR-90	Single stemmed variety with upright growth habit and glabrous leaves. Useful for seed and fodder purpose.	~13	Pakistan

Source: Ministry of Agriculture and Farmers Welfare, India http://farmer.gov.in; Seednet India Portal https://seednet.gov.in; Indian Council of Agricultural Research (ICAR), Central Arid Zone Research Institute (CAZRI), Jodhpur, India; http://mkisan.gov.in; Alternative Field Crop Manual, New Crop Resource Online, Purdue University, West Lafayette, IN, USA https://hort.purdue.edu/newcrop/afcm/guar.html; Saleem et al. (2002)

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Chapter 5
Common Bean (*Phaseolus vulgaris* L.) Breeding

Oswalt R. Jiménez

Abstract The common bean (*Phaseolus vulgaris* L.) is a grain legume species, mostly cultivated in many developing countries of Africa, America and Asia. It is considered a key crop for improving food security of people vulnerable to malnutrition. From the 1930s, common bean genetic improvement has historically been conducted by international programs in coordination with government institutions and following traditional methods. Those efforts have created successful varieties in recent decades. But, current climate change effects and the reduced adoption of adequate technologies for cultivation, have threatened common bean productivity. Probably, challenges for the next decades cannot meet using only traditional breeding. Thus, new techniques and approaches for conducting breeding should be soon adopted to obtain new varieties with broad resistance to varied biotic and abiotic stresses. When planning new breeding programs, it is important to consider the current agro-biotechnology advances in molecular markers, functional genomics, mutagenesis, tissue culture and even genetic engineering, which could improve breeding efficiency. Additionally, the conservation, utilization of genetic resources and the promotion of participatory breeding will be crucial to strengthen the least productive common bean systems. It will be important to provide varieties that respond well to agro-ecological management under an integral ecology approach. Finally, it is evident that there is still an opportunity to improve productivity by improving access and adoption of more resilient technologies. In this particular case, community seed banks can play an important role in the future.

Keywords Agro-biotechnology · Climate change · Food security · Participatory breeding · Traditional breeding

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5.1 Introduction

The common bean or dry bean (*Phaseolus vulgaris* L.), chromosome number 2n = 2x = 22, genome size ~637 Mbp (Varshney et al. 2010), is an herbaceous annual plant, cultivated worldwide by millions of small-scale farmers for the purpose of harvesting its seeds and immature pods. Mostly, bean production is for subsistence in developing countries involving farmers with low incomes and problems associated with malnutrition. This edible legume crop is recognized as a pulse crop due to the high protein and fiber content of its seeds and low fat content. Its origins extend back to the development of ancient American civilizations; where in combination with other crops such as maize (*Zea mays*), amaranth (*Amaranthus* spp.), cucurbits (*Cucurbita* spp.), tomato (*Lycopersicum esculentum*) and cacao (*Theobroma cacao*), provided food for people before Spanish colonization of the Americas after 1492 (Bukasov 1931; Dressler 1953). After that period, common bean and its cultivation practices were introduced into African and Asian countries, adapting to new crop conditions and providing important nutrition to people to the present day.

The Natural Resources Conservation Service (NRCS) classifies this species as belonging to the genus *Phaseolus*; family Fabaceae; order Fabales; sub-class Rosidae, and class Magnolipsida (USDA 2018). The genus *Phaseolus* encompasses more than 52 species distributed around the world, including wild and cultivated types (Gepts and Debouck 1991). According to FAOSTAT (2018), Asian countries harvested the greatest area in the world (15,101,109 ha; 49.33%) in 2014, followed by Africa (7,653,580 ha; 25%) and the Americas (7,512,139 ha; 24.54% of the total), respectively (Table 5.1). In the same way, the highest production was obtained from Asia (11,660,529 mt; 43.95%) followed by the Americas (7,942,764 mt; 29.94%) and Africa (6,192,711 mt; 23.34% of the total). However, yield statistics position European countries with the greatest yields (2.25 mt/ha) followed by the Americas (1.06 mt/ha).

Table 5.1 Common bean harvested area, production and yield in Africa, America, Asia, Europe and Oceania

Continent	Harvested area (ha)	Production (mt)	Yield (mt/ha)
Africa	7,653,580	6,192,711	0.81
America	7,512,139	7,942,764	1.06
Asia	15,101,109	11,660,529	0.77
Europe	311,014	701,575	2.25
Oceania	35,000	32,000	0.91
World (Total)	30,612,842	26,529,579	0.86[a]

Source: FAOSTAT (2018)

[a]average data, *mt* metric tons, *ha* hectares

Table 5.2 Common bean: world ranking of the twenty largest country producers ordered by production and indicating yields in 2016

Country	Production (mt)	Yield (mt/ha)
Myanmar	5,189,977	1.68
India	3,897,611	0.41
Brazil	2,615,832	1.01
USA	1,269,916	2.01
Tanzania	1,158,039	1.04
China	1,139,866	1.64
Mexico	1,088,767	0.69
Uganda	1,008,410	1.50
Kenya	728,160	0.62
Ethiopia	483,923	1.66
Rwanda	437,673	0.85
Cameroon	390,816	1.30
Burundi	371,892	1.78
Angola	367,255	0.44
Argentina	366,588	1.02
Korea	320,399	0.88
Belarus	277,755	2.51
Indonesia	277,408	1.16
Canada	249,400	2.26
Guatemala	247,680	0.98

Source: FAOSTAT (2018)

For 2016, statistics tabulated by country indicated that the five largest common bean producers were Myanmar, India, Brazil, USA and Tanzania (Table 5.2). Others positions in ranking of the twenty largest common bean producers are mainly represented by African and American countries. Common bean production in North America is promoted by residents coming from Latin American countries that represent the so-called *nostalgic market*. However, yield data are showed do not match with the greatest production, meaning that while some countries have relatively high production, for instance India and Mexico (3,897,611 and 1,088,767 mt, respectively), but yields are low compared with other countries.

This fact could be ascribed to the high investment of appropriate technology during production such as utilization of high-quality seeds, irrigation systems, and proper pest and diseases control during growth and development stages, appropriate plant nutrition practices, among others. Countries producing above 1 million mt, Myanmar, Brazil, USA, Tanzania, China and Uganda, also registered yields of more than 1 mt/ha. Belarus registered the highest yield (2.51 mt/ha), but its total production is small compared with other industrialized countries.

5.1.1 Importance for Human Nutrition and Food Security

The common bean is cultivated as a staple food mainly by farmers under subsistence conditions in developing countries, where it represents the main source of protein, iron and zinc to people vulnerable to malnutrition. Lareo and Gonzalez (1988) provided a valuable literature compilation of the potential benefits of incorporating beans in the human diet. That review estimates that crude protein in bean seeds is 16–30%. This protein can be divided into five main fractions: phaseolin (36–46% by weight), globulin-2 (5–12%), albumin (12–16%), prolamine (2–4%) and alkali-soluble fraction (20–30%). The same source mentions that iron content is on average 70 mg/kg and in the same way as protein, its concentrations varies depending on environmental and cultivating conditions. Protein intake, in humans, can be improved from 4.54 to 6.26% when combined in a ration 3:7 with cereals such as maize. Indeed, some nutritionists point out that common bean and maize are a perfect food combination due to amino acids complementarity, enhancing their assimilation by humans (Mora-Aviles et al. 2007).

Condensed tannin and anthocyanin content, the first affecting iron absorption and the second with antioxidant properties, have a relationship with seed coat coloration (Díaz et al. 2010a). In this sense, total phenolic content (free, soluble conjugate, insoluble bound fraction) have diverse antioxidant capacities that vary depending upon the variety, some genotypes being an important dietary sources of natural antioxidants for prevention of diseases triggered by oxidative stress (Wang et al. 2016). Recent studies suggest that peptides present in common bean seeds, specifically in non-digestible fractions, have an antiproliferative effect on human colorectal cancer cells by modifying expression of markers associated with cell cycle arrest or mitochondrial activated apoptosis (Luna et al. 2014). Additionally, common bean consumption could have the potential to reduce serum cholesterol concentrations, improving health conditions in diabetic patients and providing, in many aspects, metabolic multiple benefits for weight control as well (Anderson et al. 1999). Also, it is estimated that the consumption of partially-hydrolyzed bean seeds may provide important functional elements to protect cells against inflammation present in injured tissue or chronic disease conditions (Oseguera-Toledo et al. 2011).

Despite the natural high nutritional quality of beans, they are considered a suitable vehicle for iron and zinc biofortification (Petry et al. 2015). Thus, there have been efforts to develop and release biofortified varieties in many developing countries. These varieties have been added to local seed programs aiming to improve iron, zinc and protein intake in rural families (Saltzman et al. 2017). Many seed programs are distributing seeds from those varieties to people vulnerable to malnutrition. The release, production and distribution of common bean varieties with high protein, iron and zinc contents can be traced, following information from the centers and institutes listed in Appendix I.

5.1.2 Climate Change Scenarios and Challenges

Reliable knowledge about the occurrence of different phenomena related to the variability and changes of climate that scientists have projected for the coming decades is essential for the establishment of resilience strategies in agriculture. Here, genetic improvement of key crops including common bean in conjunction with the adoption of new management practices, will be vital to prevent global famine.

Currently, it is difficult to predict with a high level of certainty how the effects of change and climatic variability are going to affect bean production in each part of the world. Therefore, each country must carefully analyze the different projections and take corresponding measures. It is almost a shared consensus that temperatures will increase in magnitude globally with continents experiencing reduction or increase in precipitation rates (Christensen et al. 2013). Model predictions point out that East Africa could experience impacts in common bean yields from −18.1 to +23.7%. Similarly, Southern Brazil could present impacts of up to +45% and for Central America from −4 to −87% (Porter et al. 2014). The range of predictions is quite large and it limits, to an extent, the drafting of general measures per region. More valuable information about global climate change reports can be found at the Intergovernmental Panel on Climate Change's website (http://www.ipcc.ch/).

According to global climate models, CSIRO-Mk3 and MIROC-H, for the years 2050 and 2100 climate conditions are expected to be more favorable for common bean cultivation in the Northern Hemisphere, but unfavorable in the Southern Hemisphere (Ramírez-Cabral et al. 2016). Also, heat and drought stresses are considered among the foremost factors limiting common bean production under present and future projected conditions (Beebe et al. 2011; Beebe 2012; Ramírez-Cabral et al. 2016; Rodríguez and Creamer 2014). Based on those scenarios, considerable parts of Africa and Latin America, where common bean is a crucial staple food, are projected to reduce areas for cultivation because of unfavorable conditions, affecting the food security of millions of people whose survival and nutrition relies on this legume (Wortmann et al. 1998). For South America, projections indicate that in comparison with the historical period (1980–2005), climate change will make drought more recurrent, but less severe across this part of the continent (Heinemann et al. 2017).

For heat and drought conditions, a comprehensive understanding of plant behavior through the application of biophysical crop models is needed. For instance, during field experiments using common bean plants exposed to heat and drought stresses, the Farquhar-Ball-Collatz model (FBC model) was found to be reliable to predict water dynamics, plant growth and stomatal conductance in comparison with the Goudriaan and van Laar model (GvL model) (Seidel et al. 2016). It will be important to continue setting up, calibrating and validating more models to provide useful information to design experiments for plant selection under stressful conditions.

Water scarcity is a limitation for common bean, which requires at least 300–500 mm of rainfall throughout the 60–120 days of growth and developmental stages (Allen et al. 1989; White et al. 1995). In addition, periods of fluctuating rainfall can cause significant losses due to off-season rains that create conditions for the occurrence of fungal and bacterial infections, more so when they coincide with the periods of pre-harvest, harvest and drying of the grain (the latter, many times, are carried out in the open field in semiarid and inter-cropping systems). After more than nine days of heat stress (33 °C days and 27 °C nights) before anthesis, the anthers of the heat-susceptible varieties could experience indehiscent and abnormalities in pollen grains reducing seed formation and yields (Gross and Kigel 1994; Porch and Jahn 2001). It is estimated that a heat stress of 33 °C days and 30 °C nights could reduce yield components; seed number, pod number, mean seed weight and seeds per pod by 83, 63, 47 and 73%, respectively (Rainey and Griffiths 2005). When drought and heat stresses are present together, the effect can be overwhelming, generating total crop loss.

On the other hand, increased temperatures, elevated CO_2 levels and precipitation variations, conditions projected in various climate scenarios, may trigger significant changes in pathogen and pest population dynamics (Jones 2016). Thus, pest and disease populations could experience changes in population growth rates, number of generations, occurrence, interspecific interactions, virulence, balance of natural enemies and efficacy of crop protection technologies (Macedo et al. 2017; Reddy 2013; Sharma et al. 2010; Taylor et al. 2018). Under those conditions, some secondary or even tertiary pests and diseases could turn into first order, limiting control measures because of the lack of proper practices and experience by farmers and technicians to control them. Also, the dynamics of many insect vectors could become aggressive, spreading new virus strains to other locations (Jones 2016; Jones and Barbetti 2012).

5.1.3 Domestication, Selection and Early Improvements

There is extensive literature on the domestication and origin of the common bean, using different approaches, some posit common bean with a Middle American origin and another Andean. But, most researchers agree that this species was domesticated following two main events, in Middle America and the Andes. According to multiple DNA-based studies, this species was probably domesticated from wild forms around 8000–10,000 years ago (Bitocchi et al. 2012, 2013; Chacon et al. 2005; Gepts and Debouck 1991; Schmutz et al. 2014; Singh 1992). Archaeological records, DNA and morphological differences between domesticated and wild species indicate that ancient civilizations selected from wild types those plants holding more upright growth habits, shorter stem inter-nodes, completely indehiscent pods and larger seeds; ultimately accounting for current cultivated phenotypes (Gepts and Debouck 1991; Schmutz et al. 2014). Likely, the occurrence of spontaneous

mutations and natural hybridizations contributed to broaden genetic variability, crucial during human selection.

Voysest (1983, 2000) reported the early improvements of common bean carried out between 1930 and 1999. There is consensus that common bean breeding activities began on 1930s, probably simultaneously in Mexico and Brazil. There is limited historical information about the breeding process for obtaining the first varieties, but it is inferred that breeders started by improving agronomic traits of local landraces. The first varieties were named for their seed coloration and other main attributes. Later in the 1940s, the Rockefeller Foundation sponsored formal common bean breeding programs in Mexico and Colombia. The first initiatives for breeding activities in collaboration among countries were coordinated from Costa Rica, incorporating efforts from Mexico, Guatemala and El Salvador. Later in 1962 the Central American Cooperative Program for Common Bean Breeding (PCCMF, Spanish acronym) was created through the Centro American Cooperative Program for Food Crop Improvement (PCCMCA, Spanish acronym). Between 1950 and 1970, common bean programs operating locally in Mexico, Colombia and Costa Rica, were strengthened through international cooperation which provided training for young researchers from different Latin American countries. The Inter-American Institute of Agricultural Sciences (IICA, Spanish acronym) and Pan-American Agricultural School (Zamorano, Honduras) both played important roles in the development common bean in the region. Around 91 varieties were evaluated during that period. In 1973, the International Center for Tropical Agriculture (CIAT, Spanish acronym) inaugurated its Grain Legume Program through an international symposium entitled: Potential of Grain Legumes. Since then, CIAT has provided a leadership tradition that persists in common bean breeding, with remarkable worldwide achievements to the present.

In 1980, the Bean/Cowpea Collaborative Research Support Program was established for the purpose of improving the nutritional situation of people suffering from food scarcity and enhancing human capacities for bean and cowpea research. Four American universities took part in important projects at that time. The program contributed significant new varieties, improving nitrogen fixation by symbiosis with rhizobia, improving nutritional quality and digestibility of seeds, and conferring resistance to heat, drought and viral infections. Kelly and Cichy (2013) document that there were six public bean breeding programs at major land grant universities, four programs that focused on bean genetics and pathology and five private companies actively working on bean breeding in the USA. Singh et al. (2007) and Teran et al. (2009) reviewed the bean breeding improvements in the western USA over 56 years, documenting 34% genetic gains, increasing seed size and incorporating some degree of resistance to diseases in most varieties. In Brazil, the Brazilian Agricultural Research Corporation (Embrapa, Portuguese acronym) Rice and Beans, has coordinated, since 1974, a program for the genetic improvement of carioca-type common bean for the entire country. According to Faria et al. (2013) and Moreira et al. (2010), between 1984 and 2010 this program released around 50 new varieties (mostly using pedigree method) at an average rate of 1.9 varieties per year, showing significant genetic progress in terms of grain yield (0.72% per year),

plant architecture (2%), resistance to lodging (2%) and quality of grains (2.4% per year). At present, Embrapa Rice and Beans continues to provide bean farmers with highly-productive cultivars with high-quality grains.

In Tanzania, the first bean improvement program was initiated at Tengeru Agricultural Research Institute (TARI), near Arusha, in 1959. Subsequent events were summarized by Allen et al. (1989) and Hillocks et al. (2006) in a review of common bean breeding activities in Tanzania 1959–2005. A total of 82 accessions were introduced into the breeding program at TARI from around the world, 1960–1961, which released the first rust (*Uromyces appendiculatus*) resistant varieties, Tengeru 8 and Tengeru 16. In the 1970s the first National Bean Improvement Program in Tanzania began breeding to improve the quality and yield of beans. In 1975 a total of 1046 germplasm lines had been collected at three centers; Uyole Agriculture Center in the south, Ilonga Agricultural Research Institute in the center and Lyamungu Agricultural Research Institute (LARI) in the north. Later, regional networks and international programs played an important role to enhance bean-breeding activities, CIAT in Colombia being an important partner. Today, breeding programs are working collaboratively to overcome common limitations in around 30 African countries. Detailed information is available at web pages shown in Appendix I.

5.2 Cultivation and Traditional Breeding

The genetic improvement of key crops can provide dependable solutions to the problems resulting from climate change only if the varieties created are released in conjunction with the most appropriate forms of sowing and field management to allow exploiting all the attributes that plant breeders have identified throughout the experimental phase.

5.2.1 Current Cultivation Practices

In developing countries, the common bean is cultivated in semiarid and subsistence intercropping systems; small and large monoculture are also present in countries with higher incomes (Singh 1992). Regardless of the diversity of cropping systems, plant breeding can still play an important role to improve productivity, in semiarid and subsistence intercropping systems, which includes poor farmers in the developing countries (Waldman et al. 2014).

It is indisputable that despite the fact that regional breeding programs have released successful varieties, there is still low productivity in relation to the actual potential of the species. This situation is caused in part by the low utilization of high-quality seeds in developing countries. Diverse reports from Africa and Central America point out that typical bean farmers use their own seeds for sowing (Asfaw

et al. 2013; Goettsch 2016; IICA 2009; Katungi et al. 2011; Opole et al. 2003; Wortmann et al. 1998). In Central America, for instance, it is estimated that only 14% of common bean farmers use certified seeds for production, the remaining area is sown with self-produced seed of suboptimal quality (IICA 2009).

Some seed programs have implemented a modality called *Community Seed Banks* (CSBs) with the purpose of increasing the availability of adapted seeds at lower costs, compared with certified seeds. According to FAO (2014) Community Seed Banks are farmers' organizations that store and manage seeds, aiming to provide community members with seeds to use, which are obtained from the farmers in the community. Those organizations provide seeds of improved varieties, landraces and old cultivars to the region, receiving in some successful cases, a sort of technical supervision and following quality standards from non-governmental and governmental institutions. The results and positive impacts of these initiatives have been documented, highlighting successful cases around the world (Coomes et al. 2015; FAO 2014; Katungi et al. 2011; Vernooy et al. 2015, 2017). Nevertheless, it is important that CSBs take into account some technical principles of seed production; otherwise this mechanism could be distributing low-quality seed and spreading important seed-borne pathogens (Marcenaro and Valkonen 2016). The use of common bean seeds with low vigor could reduce yield by up to 20% (Mondo et al. 2016); whereas the use of proper plant densities in semiarid and subsistence systems can increase bean yield by 30–70%, also avoiding costs associated with weed management (Dusabumuremyi et al. 2014).

It is known that many agricultural research institutes have published local technical bulletins, guidelines and booklets about appropriate practices for common bean cultivation, as outputs of different regional projects. More detailed information about technical documentation is available at the international institution websites listed in Appendix I. Nonetheless, despite all available documentation, the adoption and implementation of best practices is still too low (Goettsch 2016; Opole et al. 2003). Most common bean production in developing countries is managed using only minimal inputs. That means, farmers after sowing, carry out weed control, fertilization, pests and disease management, pre-harvest, harvest and storage, using scarce resources and family labor, limiting the real potential of varieties developed by international programs which respond best to optimum management practices (Goettsch 2016; Opole et al. 2003). In addition, common bean can be found prospering in mixture systems with other crops (Singh 1992; Woolley and Davis 1991; Woolley et al. 1991). According to Argaw and Muleta (2017) and Taylor et al. (1983) the simple adoption of the best-adapted variety, use of quality seed and the inoculation of the seed with an appropriate rhizobium strain could easily double yields; adoption of complete management packages could improve common bean production even more. On the other hand, in industrialized countries the suitable utilization of technology has prompted high yields and good economic profitability for special markets as in North American, for instance, where common bean systems are based on monoculture with the use of complete technological packages (Kelly and Cichy 2013; Thung 1991).

It is also important to consider the increasing interest in the use of bio-inputs (beneficial biological agents, fungi, bacteria) for fertilization and crop management of pests and diseases. Far from trying to create an economic dependence, bio-inputs could be produced at the laboratory level, as well as at the farm level by artisanal methods, reducing the costs associated with their wide use, all this under an integral ecology approach.

5.2.2 Improvement Strategies, Methods and Limitations

International breeding programs work on a regional framework, centralizing the first stages of breeding at headquarters locations of international centers and institutions and employing specialized expertise. Thereafter, activities are coordinated with national institutes of agriculture research (NIARs) in target countries, to carry out evaluations and yield trials under different environmental conditions until release of a new variety (Fig. 5.1). This approach optimizes economic and human resources. However, it is important to point out that there are some NIARs that have a robust research agenda, developing most common bean breeding activities at the local level.

Since the first formal common bean breeding programs began in the 1930s, most activities have involved phenotypic evaluations and pedigree information. These programs began by making manual crossings between or among elite parents holding desirable characteristics. From 20 to 200 crosses could be performed to obtain a desirable number of individuals at the F_1 generation, but it would depend on economic resources and facilities (Acquaah 2007, 2012). Common bean breeders could improve the success percentage of effective crosses by adopting procedures that reduce minimum damage to floral buds, increase seed setting and confirm successful hybrid F_1 plants by using DNA markers (Jiménez and Korpelainen 2013; Kelly and Cichy 2013).

Individual self-pollinated species are highly homozygous in most of their loci, although heterogeneous when compared among populations. This means that via directed crosses it is possible to reshuffling allelic combinations to search for new promising genotypes, to be fixed in subsequent generations. In this sense, crossing has become a crucial approach for obtaining working populations that permit the greatest possible genetic variability.

It is important to highlight the limitation of plant breeding processes based mainly on mass selection, since the opportunity to identify new and useful allelic combination is very small compared to the segregating populations obtained from crosses (Jiménez and Korpelainen 2013). Conducting crosses requires expertise and resources, but the benefits are significant since by choosing suitable parents it is possible to create thousands of divergent pure lines. There are four different gene pools for the common bean showing a certain degree of cross incompatibility: primary gene pool comprises both *P. vulgaris* cultivars and wild relatives from that species; secondary gene pool that encompasses *P. coccineus*, *P. dumosus* (=*P.*

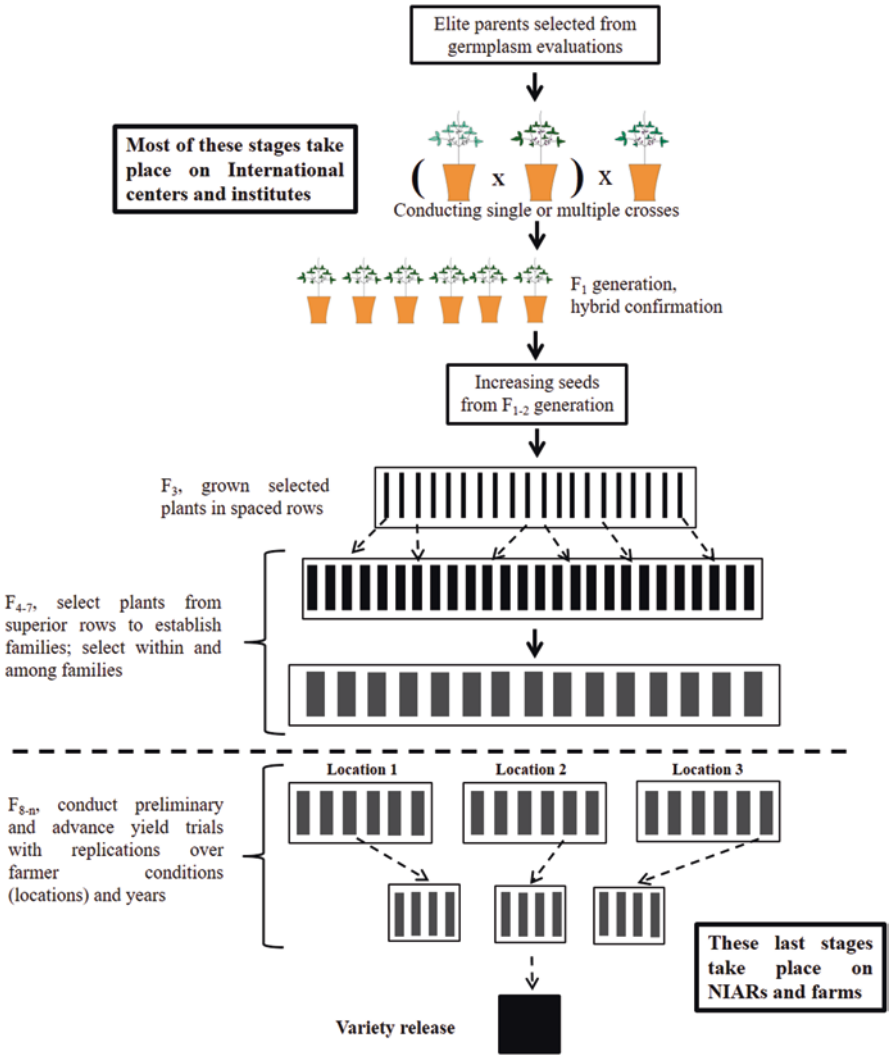

Fig. 5.1 Flowchart showing the basic steps in common bean breeding. The dotted line separate activities carried out in international centers from those on NIARs

polyanthus), and *P. costaricensis*; tertiary gene pool including *P. acutifolius* and *P. parvifolius*; and quaternary gene pool that counts *P. lunatus*, *P. carteri*, *P. filiformis* and *P. angustissimus* (Pathania et al. 2014). For the utilization of genes from different gene pools, specifically for crosses of *P. acutifolius, P. coccineus*, *P. costaricensis* and *P. polyanthus* with the common bean, it is necessary to improve crossing performances by tissue culture techniques for the rescue of embryos in early developmental stages (Andrade-Aguilar and Jackson 1988; Geerts et al. 2002; Ivančič and Šiško 2003; Mbogo 2007; Mejía-Jiménez et al. 1994; Pathania et al. 2014;

Porch et al. 2013). These crosses between different species have allowed incorporating valuable alleles for conferring resistance to biotic and abiotic stresses and nutritious value in important breeding lines. Protoplast fusion techniques have also been used to hybridize different *Phaseolus* species (Geerts et al. 2008). However, the recalcitrant nature of the common bean has limited its use due to the very low regeneration rates as addressed in Sect. 5.5.1. It is important to point out that recent efforts could improve plant regeneration rates in the coming years.

In the F_2 generation, plants are grown in greenhouse to increase the quantity of seeds obtained from each F_1 plant. From F_2 generation onwards, selection could adopt different pathways depending upon the selection method used. Acquaah (2007, 2012) provides a comprehensive description and examples of different selection methods that can be used to perform selection from the F_2 generation until release of a new variety. However, most current common bean varieties have been obtained using the pedigree method (Kelly and Cichy 2013; Moreira et al. 2010). In some cases, breeders may innovate in combining the best attributes of two or more methods as one, obtaining benefits in genetic gains.

Most developing countries receive pure lines or families (F_8–F_{12} generations) from international breeding programs to be incorporated into local research projects in NIARs. Thus, once common bean nurseries are received, the latest stages of selection are focused on comparisons among families and lines and not on selecting individuals within populations. Thus, there is no chance to improve important traits in these genetic materials. These field trials are conducted under farmer field conditions to ensure profitability and acceptability by consumers as well. Unfortunately, each NIAR releases a new variety applying their own nomenclature, causing confusion and making the identification of varieties difficult because the same genetic material can be in more than one country, but holding different names.

It is evident that even under this efficient approach, there is not enough genetic variability in advance genetic materials to confront new challenges, because it derives from a narrow genetic base (Pathania et al. 2014). The wide gamma of genetic diversity documented in various marker-based studies has not been exhausted by all breeding programs (see Sect. 5.3.1 for references). All this germplasm could conserve useful allelic variation to be incorporated into breeding programs. But, this option has been limited by reduced funding, inadequate infrastructure (laboratories, greenhouse) and lack of skilled human resources to conduct those activities in countries of origin.

Most breeding activities have used traditional methods, without actively exploiting all available biotechnological tools to strengthen bean breeding programs and increase efficiency (Aragão et al. 2013; Dwivedi et al. 2006; Ender et al. 2008; Gupta et al. 2010; Kumar et al. 2011; Mahuku et al. 2004; Miklas et al. 2000, 2006; Oliveira et al. 2005; Pasev et al. 2014; Schneider et al. 1997; Varshney et al. 2010; Yu et al. 2000; Zargar et al. 2017). Moreover, breeding activities in a good number of NIARs have not strengthened local breeding activities aiming to complement regional efforts. For instance, it would be practical to incorporate new alleles into old but successful commercial varieties, conserving desirable characteristics by backcrossing. This could be considered a derived variety according to the

International Union for the Protection of New Varieties of Plants (UPOV, Spanish acronym) (UPOV 1991, 2017). Also, it could be important to consider maintenance breeding as an option to overcome loss of adaptation of some old varieties (Peng et al. 2010). As the success of symbiosis of common bean and bio-inputs increases significantly yields (Bennett et al. 2013; Blair 2013; Dall'Agnol et al. 2013; Kawaka et al. 2014; Maougal et al. 2014; Snoeck et al. 2003; Weisany et al. 2016) and it depends on variety and microorganism strain correspondence (Samago et al. 2017), it is important to consider the inclusion of this factor/variable during selection as the response of a variety to management using bio-inputs for fertilization, pest and disease control. All these strategies seem promising because they decrease production costs, increase soil fertility, disrupt the life cycles of pests and diseases and improve resilience under the climate change context.

On the other hand, recently, participatory breeding has risen forcibly in Latin American and African countries in response to the necessity of providing well-adapted varieties to local conditions. Basically, this approach is conducted starting with planning workshops led by breeders, technicians and some well-trained farmers. During these workshops, farmers in coordination with breeders define an ideotype as a target, holding the characteristics depending on environmental conditions and farmers' preferences. Thereafter, farmers collect all the available germplasm from the community or abroad; this collection may include landraces, old cultivars and improved varieties. In practical terms, the initial genetic material as a population is highly heterogeneous, but homozygous at the same time. Most participatory breeding programs are carried out using mass selection on homozygous populations, but there are some experiences where populations obtained from crosses are also used (Almekinders 2011; Asfaw et al. 2012). Because the mass-selection method is not efficient for quantitative traits with low heritability, such as yield, it is possible to improve the performance of participatory breeding at initial stages by adopting other selection methods, for instance, mixed-model selection under augmented block design that has been used in different species with successful results (Aruna and Audilakshmi 2008; Balestre et al. 2013; Gonçalves-Vidigal et al. 2008; Oliveira et al. 2012; Piepho et al. 2008; Upadhyaya et al. 2009). Participatory initiatives are a good source of adapted varieties for farmers when combined with seed multiplication at CSBs. Unfortunately, these kinds of varieties have not found a place in current seed legislations in most countries; consequently, seed production following formal mechanisms is not possible for those varieties, despite their high-resilience potential (Dawson and Goldringer 2012).

5.2.3 Role of Biotechnology

Present advances in plant biotechnology and recent reference genome and genome-wide analysis for common bean have made available different methods with high potential to be used for breeding (Schmutz et al. 2014), refining the incorporation of key traits, the performance of selection programs or for identifying promising

parents for crosses. Marker-assisted selection (MAS), genomic selection using single nucleotide polymorphisms (SNPs), genome editing using CRISPR/Cas system, plant transformation, in vitro culture, are some examples of those developments (Aragão et al. 2013; Dwivedi et al. 2006; Ender et al. 2008; Gupta et al. 2010; Kumar et al. 2011; Mahuku et al. 2004; Miklas et al. 2000, 2006; Oliveira et al. 2005; Pasev et al. 2014; Scheben et al. 2017; Schneider et al. 1997; Varshney et al. 2010; Yu et al. 2000; Zargar et al. 2017). But, the use of these approaches have been quite limited in current international programs, because of high costs (Blair et al. 2007; Varshney et al. 2005) and the misconception that biotechnology tools frequently are confused with genetic engineering or transgenics by farmers' organizations. Although Sect. 5.2.2 stressed the importance of exploring and exploiting all the available germplasm, in some cases recruiting novel alleles by phenotypic methods could be too slow, so new traits related to resistance to drought, heat, pests and diseases may be supplied through biotechnology (Svetleva et al. 2003); therefore, it is economically reasonable to balance the time and cost for certain traits that biotechnology could incorporate into new varieties to cope with new future challenges.

5.3 Germplasm Biodiversity and Conservation

Plant breeders evaluate large numbers of accessions looking for new sources of genes from different gene pools that, together with seed bank curators and other experts, can identify and study their potential for genetic improvement. Without genetic variability the work of the breeder is severely limited, since the selection process is a game of numbers whose richness significantly increases the chances of finding something new and useful. This is why ex situ and in situ collections represent a key element of any plant breeding process.

5.3.1 Germplasm Diversity and Phylogeny

The common bean is a species that exhibits high phenotypic variability of most of the 90 traits commonly suggested for phenotypic studies and variety description (Muñoz et al. 1993). Seed characteristics; size, color and shape are the most relevant and visible trait when identifying a variety and it determines in some countries the market value of beans, because of consumer preferences (Fig. 5.2). Singh (1989) in one of the most extended studies, examined 18,000 accessions from the CIAT seed bank at two different locations in Colombia, describing relevant phenotypic diversity in populations collected from different gene pools. That study divided germplasm into a total of six gene pools from Middle American and four gene pools from South American centers of domestication. In a more recent study, Rana et al. (2015) described the characterization of a collection of 4274 accessions originating from

Fig. 5.2 Phenotypic diversity for seed traits in *Phaseolus* collections of Mesoamerican origin. Photos: Rows (**a** and **b**) courtesy of National Institute of Forestry, Agriculture and Livestock Research (INIFAP), Mexico. Rows (**c** and **d**) courtesy of National Center of Genetic Resources (CNRG), INIFAP, Mexico. Scale in centimeters (cm)

58 countries using 22 phenotypic traits measured during two years, finding high phenotypic diversity for traits such as leaf length, leaf width, pod length, pods per plant, seeds per pod and 100-seed weight.

Blair et al. (2009) analyzed the level of genotypic diversity in a collection composed of 604 accessions collected from different countries and continents, indicating on average 18.4 alleles per locus. In primary and even secondary centers of origin in Africa, America, Asia and Europe, analysis using different kind of markers revealed a high level of genetic diversity, suggesting spontaneous hybridizations among gene pools and mutations playing a relevant role in fixing new allele variants after introduction from America (Angioi et al. 2010; Asfaw et al. 2009; Ávila et al. 2012; Blair et al. 2006, 2009, 2012; Burle et al. 2010; Cabral et al. 2011; Díaz et al. 2010b; Fisseha et al. 2016; Gómez et al. 2004; Jiménez and Korpelainen 2012; Kwak and Gepts 2009; McClean et al. 2012; Nemli et al. 2017; Raggi et al. 2013; Santalla et al. 2010; Sharma et al. 2012; Tiwari et al. 2005; Xu et al. 2014; Zhang et al. 2008).

5.3.2 Genetic Resources Conservation Approaches

Common bean genetic resources are conserved ex situ, in seed banks and in situ on farms around the world. The first strategy allows conserving (intact) representative seed samples in cold rooms at low or ultra-low temperatures (from −20 to +4 °C) for long time periods, until physiological quality declines by natural aging. On the other hand, in situ conservation is conducted on farms following normal production systems and allowing the varieties to continue to evolve and change. There is consensus that the best approach is to combine efficiently both strategies, taking advantage of both in situ and ex situ systems as an integrated approach.

The Seed Bank of CIAT located in Palmira, Colombia holds the biggest common bean collection in the world (Johnson et al. 2003). This collection consists of around 37,390 accessions, with 441,225 samples distributed for breeding and research purposes to 105 countries. About 71.6% of the distributed samples have been used actively in breeding programs conducted by CIAT, 15.5% have been requested by the National Agricultural Research System, USA (NARS), 10.4% by various universities and the remaining 2.5% has been distributed to private companies and other applicants (CIAT 2018). It is estimated that over 70% of the value of increased common bean production is due to the use of imported varieties derived from breeding programs using the CIAT bean collection (Johnson et al. 2003). There are also important *Phaseolus* collections in international centers and research institutes elsewhere in the world.

On the other hand, in situ conservation of the common bean germplasm has relevance as a policy for improving crop resilience under climate change conditions in different countries (Coomes et al. 2015; FAO 2014; Katungi et al. 2011; Vernooy et al. 2015, 2017). In addition to producing seeds, CSBs also contribute to the conservation of genetic diversity on farms and ensure continuity. Under on-farm conservation, biotic and abiotic factors, in combination with the systematic processes underlying phenotypes, creating genetic divergence at the subpopulation level with significant implications for conservation (Thomas et al. 2015; Tiranti and Negri 2007). Studies in Uganda point out that the production of several landraces and improved varieties in mixed cropping systems is important for pest and disease management (Mulumba et al. 2012; Ssekandi et al. 2016).

5.3.3 Cytogenetics

The common bean is a diploid species with chromosome number 2n = 2x = 22 (Fig. 5.3) and genome size ~ 637 Mbp (Varshney et al. 2010). There are 52 species in the genus *Phaseolus*, most have the same chromosome number with the exception of *P. leptostachyus* (2n = 20) (Delgado-Salinas et al. 2006; McClean et al. 2008). Some minor variations between *P. vulgaris* and *P. lunatus* genomes are

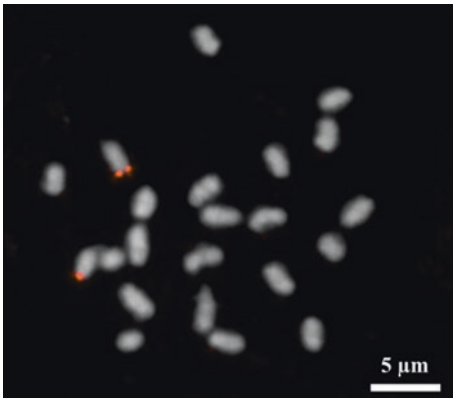

Fig. 5.3 Common bean chromosomes showing in situ localization of the single-copy BAC (Bacterial Artificial Chromosome) 224I16 (red) on chromosome pair 9 of *Phaseolus vulgaris*, cultivar BAT93. Chromosomes are counter-stained with DAPI (4′-6-diamidino-2-phenylindole) and visualized in gray. Photo: Courtesy of Andrea Pedrosa Harand and Artur Fonseca, Federal University of Pernambuco, Brazil

ascribed to pericentric inversions on chromosomes 2, 9 and 10 during evolution of the genus (Bonifácio et al. 2012). Aneuploidy probably has been important during the evolution of the karyotype (Mercado-Ruano and Delgado-Salinas 1998). The common bean genome is composed of 52% euchromatin, 31% pericentromeric heterochromatin, 5% ribosomal DNA (rDNA) and 12% centromeric heterochromatin (Fonseca et al. 2010). In comparison with other legumes, with the exception of soybean, the common bean shares same or similar chromosome number with cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), rice bean (*Vigna umbellata*), pigeon pea (*Cajanus cajan*) and hyacinth bean (*Lablab purpureus*) (McClean et al. 2008).

5.4 Molecular Breeding

The molecular breeding of economically-important crops has attained great relevance in the last decade. Although the common bean is a crop which has had fewer efforts and resources applied to it in recent years, several research groups have made important discoveries. These have revealed the potential of marker-assisted selection and the opportunity to influence genetic improvement via genetic expression and metabolic processes which are involved in yield formation under stress conditions. As well, advances in bioinformatics and the development of platforms in cyberspace have made it possible to share applications and databases within the scientific community.

5.4.1 Molecular Marker-Assisted Breeding

Molecular markers and molecular genetic linkage maps are needed to conduct MAS (Acquaah 2007, 2012; Varshney et al. 2005). The first linkage maps for common bean were constructed using few linkage groups and included only genes controlling seed coloration and patterns (Basset 1991). Subsequently, advances in DNA markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), SSRs and SNPs provided more information for fine mapping of divergent inbred populations (Bassi et al. 2017; Blair et al. 2011; Briñez et al. 2017; Chen et al. 2017; Cordoba et al. 2010; Cortes et al. 2011; Ferreira et al. 2010; Galeano et al. 2011; Gonzalez et al. 2017; Goretti et al. 2013; Gujaria-Verma et al. 2016; Hanai et al. 2010; Kelly et al. 2003; Song et al. 2015; Valdisser et al. 2017; Vidak et al. 2017). The integration of an intra-gene pool linkage map towards a consensus linkage map is an important task to fill in gaps and also to strengthen synteny studies (Galeano et al. 2011; Song et al. 2015; Yuste-Lisbona et al. 2012). Today, there are a significant number of available markers for this species; for instance, more than 2000 SSRs markers derived from genomic and genes sequences are available for common bean (Müller et al. 2014).

Nonetheless, the use of MAS has been limited for common bean in comparison with other legumes. Most experiments have focused on improving resistance to fungal, bacterial and virus diseases (Beaver and Osorno 2009; Blair et al. 2007; Jiménez 2014; Kumar et al. 2011; Mukeshimana et al. 2005; O'Boyle et al. 2007; Oliveira et al. 2005; Rocha et al. 2012; Souza et al. 2014; Yu et al. 2000) and for selection of genotypes under drought conditions (Schneider et al. 1997) with successful results in terms of genetic gains. However, despite experimental advances, the practical use of MAS and genomic-assisted selection in international and regional breeding programs is low because of the high costs associated with the screening of large numbers of individuals at each generation (Blair et al. 2007; Varshney et al. 2005). But, considering their evident advantages, compared with traditional selection and the efforts to reduce costs and to obtain new varieties possessing novel traits, these techniques should be adopted in the future. As participatory breeding is increasing in many developing countries, with promising results, it will be important to make efforts to incorporate marker-based tools into participatory breeding to strengthen the emergence of that approach. Figure 5.4 illustrates the practical utilization of molecular markers to identify alleles for virus resistance in F_{3-4} plants.

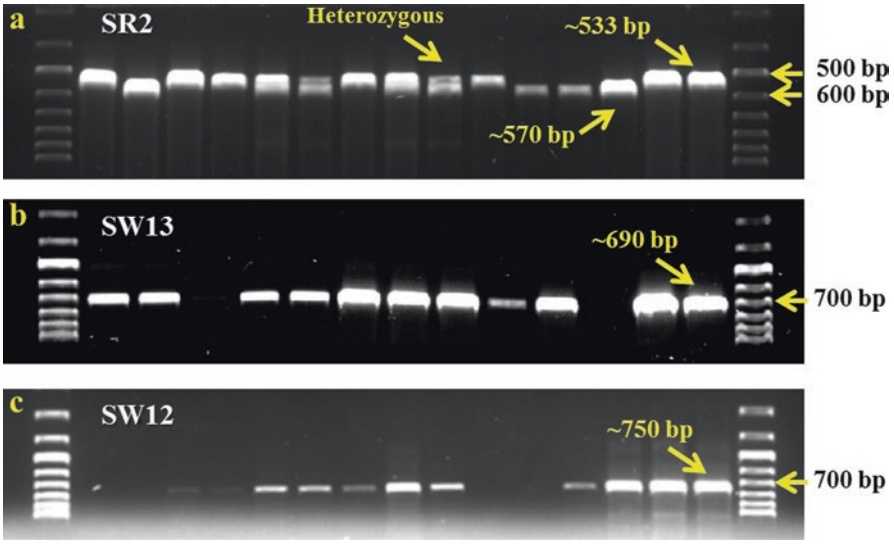

Fig. 5.4 Plant screening in F_{3-4} generation to confirm resistance to viruses. (**a**) The 533-base pairs (bp) DNA fragment at codominant SCAR marker SR2 suggests linkage to gene *bgm-1*(resistance) and the 570-bp fragment to susceptibility (Jiménez 2014), (**b**) DNA fragment 690-bp confirms the presence of gene *I* dominant for resistance to bean common mosaic virus (BCMV), (**c**) Allele 750-bp for locus SW12 confirms a variant resistance to BGYMV ascribed to major QTL obtained from variety DOR364

5.4.2 Functional Genomics

Further genetic improvement of the common bean will need a more fundamental understanding of the genetic principles of how this species responds to biotic and abiotic stresses (Schmutz et al. 2014). Despite the very incipient advances in common bean functional genomics, there have been some recent studies focused on providing insights into common bean response to drought, virus infections, aluminum toxicity, phosphorus (P-) starvation and symbiosis with nitrogen-fixing bacteria (Aparicio-Fabre et al. 2013; Formey et al. 2016; Martin et al. 2016; Mendoza-Soto et al. 2015; Nova-Franco et al. 2015; Ramírez et al. 2013; Wu et al. 2016).

In common bean-*Rhizobium* symbiosis, it was recently revealed that posttranscriptional regulator miR172c (miRNA172) plays an important role in silencing transcription factor AP2–1 (APETALA2), inducing positive effects such as improved root growth, increased rhizobia infection, increased expression of early nodulation, autoregulation of nodulation genes, improved nodulation and nitrogen fixation in common bean plants (Nova-Franco et al. 2015). As well, the study of

six miRNAs, including novel miR-RH82, involved in regulation of nodulation factors for early nodulation events in common bean roots, provided a better understanding of the role of miRNAs in rhizobia-common bean symbiosis (Formey et al. 2016). Nanjareddy et al. (2016a) discovered the role of TOR (the target of rapamycin) protein kinase during the common bean-*Rhizobium tropici* symbiotic interaction by means of posttranscriptional gene silencing of TOR using RNA interference, demonstrating that these genes are involved in lateral-root elongation and root-cell organization and also alters the density, size and number of root hairs. Improved expression of TOR ATG genes (in TOR-RNAi roots) indicated that TOR plays a role as well in even the recognition of *Rhizobium* as a symbiont. In a recent study, Arthikala et al. (2018) demonstrated through analyses using PvBPS1-RNAi transgenic roots that PvBPS1 genes (responsible of rooting and meristem formation) are critical in the induction of meristematic activity in root-cortical cells and in the establishment of nodule primordia during common bean-*Rhizobium* symbiosis.

For plant nutrition, the regulatory mechanisms of root response to aluminum toxicity (under acid soils) involve 14 up-regulated miRNAs along other regulators, suggesting that the participation of miR164/NAC1 (NAM/ATAF/CUC transcription factor) and miR393/TIR1 (TRANSPORT INHIBITOR RESPONSE 1-like protein) in auxin and of miR170/SCL (SCARECROW-like protein transcription factor) in gibberellin signaling are key for response and adaptation to this abiotic stress (Mendoza-Soto et al. 2015). Similarly, it has been inferred that the jasmonate-signaling pathway involving PvTIFY genes might be relevant in regulating common bean response and adaptation to phosphorus starvation (P-starvation) stress (Aparicio-Fabre et al. 2013). In this sense, changes in three bases of the binding site of PvPHO2 one, a negative regulator of PvPHR1 transcription factor signaling pathway that encodes an ubiquitin E2 conjugase (that promotes degradation of P-responsive proteins), are responsible for tolerance to P-starvation in some genotypes (Ramírez et al. 2013).

For drought stress, Wu et al. (2016) studied the plant-specific transcription factors CUC2 (NAC) genes that constitute with NAM and ATAF1/2 the largest families of plant transcription factors. They identified a nonredundant set of 86 NAC genes related to drought response in common bean, displaying phylogenetic relationships, conserved motifs, gene structure and expression profiles. These findings will accelerate functional genomics studies and molecular breeding programs, providing a new resource for molecular breeding even in other crops.

For plant defense, Martin et al. (2016) experimented with the transcriptional responses of a widely susceptible variety of common bean to two bean common mosaic virus (BCMV) strains (with moderate and severe symptoms) finding different transcriptome responses and large differences in splicing forms, and pathway specific expression patterns. There have been numerous studies and reviews aiming to reveal and make available useful information about the gene expression related to different stresses, suggesting the possibility of strengthening breeding programs and solving future challenges using transcriptomics and proteomics-based strate-

gies (Iñiguez et al. 2017; Jha et al. 2017; O'Rourke et al. 2014; Schmutz et al. 2014; Vlasova et al. 2016; Zargar et al. 2017).

5.4.3 Bioinformatics

There are various websites with valuable information concerning functional genomics freely available to the common bean community. For example, the *Phaseolus vulgaris* Gene Expression Atlas (PvGEA) (http://plantgrn.noble.org/PvGEA/). There, researchers can query gene expression profiles of a gene of interest, search for genes expressed in different tissue, or download the dataset in a tabular form (O'Rourke et al. 2014). Also, the database PvTFDB (https://www.multiomics.in/PvTFDB/) contains 2370 putative transcription factors gene models in 49 transcription factor families, including sequence data, functional annotation, SSRs with their primer sets, protein physical properties, chromosomal location, phylogeny, tissue-specific gene expression data, orthologues, cis-regulatory elements and gene ontology assignment. The *Phaselusgenes* is another database developed by University of California Davis (http://phaseolusgenes.bioinformatics.ucdavis.edu/) identifies and explores markers, quantitative trait loci (QTLs), and SSRs region information for common bean. Finally, there are many other resources in international institutions, not developed exclusively for common bean, but that provide useful information for breeding purposes.

5.5 Genetic Engineering

Genetic engineering, although much debated in some countries, has provided important achievements to increase yields in important crop species such as maize, soybean (*Glycine max*) and cotton (*Gossypium* spp.). Research teams worldwide have made advances in the modification of traits of great importance in response to different stresses linked to climate change. In particular, the genomic correspondence of soybean with common bean opens the possibility of taking advantages of those advances in the future.

5.5.1 Transformation and Regeneration Methods

The *Agrobacterium tumefaciens*-mediated transformation has been employed in many studies and using different common bean tissue (epicotyl containing seedling, mature seed embryos, cotyledonary node and embryonic axis explants, primary leaf explants, stem sections) obtaining genotype-dependent results (Amugune et al. 2011; Collado et al. 2016; Mukeshimana et al. 2013; Singh 2016). Five common

bean varieties were transformed via the biolistic bombardment of the apical shoot meristem primordium in order to incorporate selectable markers and genes for HVA1 protein which confers drought resistance by increasing root lengths in transgenic plants (Kwapata et al. 2012). Electroporation method (single pulse of 260 ms at field strength of 225 $V.cm^{-1}$) was applied to intact embryonic axes, confirming good results in different varieties through the β-glucuronidase (GUS) reporter gene (Dillen et al. 1995). Rech et al. (2008) designed a protocol by combining resistance to the herbicide Imazapyr [IUPAC name = 2-(4-Methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl)pyridine-3-carboxylic acid] as a selectable marker, multiple shoot induction from embryonic axes of mature seeds and biolistic techniques, obtaining average frequencies (the total number of fertile transgenic plants divided by the total number of bombarded embryonic axes) of producing germ-line transgenic bean plants of 2.7%, employing 7–10 months. In a recent study, Singh (2016) tested infecting with *Agrobacterium tumefaciens* (strain EHA 101, harboring GUS intron plasmid) cotyledonary node and embryonic axis explants, without any successful results. But, using primary leaf explants under Murashige and Skoog (MS), B5 vitamin and different growth hormones concentrations it was possible to recover full transgenic callus and transformed roots from this tissue with a transformation frequency of 7.5%. Nanjareddy et al. (2016b) isolated protoplasts from different tissues and transformed them using a polyethylene glycol-mannitol magnesium (PEG-MMG)-mediated transformation method with results reported by GUS assays and RT-qPCR analysis of protoplasts. Interestingly, sonication-assisted *Agrobacterium*-mediated transformation, for the leaf disc infiltration of common bean transformed 60–85% of the cells in a given area of the leaf surface, resulting in 90% of transformation efficiency.

The common bean is considered a recalcitrant species, but it is not known if it is really resistant to regeneration or transformation due to an absence of indigenous competition or if the best technique has not yet been found (Hnatuszko-Konka et al. 2014; Veltcheva et al. 2005). However, most experiments have established that the plant regeneration protocol for this species is genotype-dependent (García et al. 2012; Martínez-Castillo et al. 2015; Mukeshimana et al. 2013). Direct-shoot regeneration (without intermediate callus) using the transverse thin cell layer method with special culture conditions achieved 100% well-developed shoots and the regeneration of complete and fertile common bean plants (Cruz de Carvalho et al. 2000; Veltcheva et al. 2005). Also, the use of somatic embryogenesis based upon the use of benzyl-amino-purine (BAP) and adenine (A) coupled with osmotic stress (sucrose 12% w/v, 0.5 M) adaptation, instead of somatic embryogenesis response, that is induced by auxins, induced up to 25% complete and fertile plants in a non-genotype-dependent protocol (Cabrera-Ponce et al. 2015). Shoot induction of embryonic axes using MS culture media with B5 vitamin and BAP or Thidiazuron provided differentiated results with two varieties (Martínez-Castillo et al. 2015). Regeneration capacity of different tissues were tested on 30 media, each containing MS medium and different combinations of hormones, confirming the recalcitrance of common bean. But, better results were reported when using embryo axis explants, although optimal protocol was genotype-dependent (Mukeshimana et al. 2013).

Also, embryonic axes were cultured in MS medium containing different BAP and A concentrations, resulting in full plant regeneration up to 83% with variety identity confirmed using AFLP markers (Delgado-Sánchez et al. 2006). Embryos extracted from sterilized mature seeds and cultured in Gamborg media, containing BAP and A, showed a good differentiation of cells like bud clusters at the internodal segment of the embryo axes with up to 93% full plant regeneration (Quintero-Jiménez et al. 2010). Kwapata et al. (2010) evaluated 63 different media combinations of cytokinins and auxins for in vitro regeneration of multiple shoots and somatic embryos for ten varieties, reporting promising results for a specific media combination. It is evident that a specific protocol has to be fine-tuned in order to improve the plant regeneration for a specific variety.

5.5.2 Enhanced Traits and Transgenic Varieties

According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA 2018) only one transgenic event for common bean is recorded with the name Embrapa 5.1, whereas for soybean there are 37 events. Embrapa 5.1 was engineered using an RNA interference constructed to silence the sequence region of the AC1-viral gene of bean golden mosaic virus (BGMV), generating a highly-resistant transgenic common bean variety (Aragão et al. 2013; Bonfim et al. 2007). During the experimental phase, 18 transgenic common bean lines were obtained with an intron-hairpin construction that induces posttranscriptional gene silencing against the AC1 gene. As result of that phase, approximately 93% of plants from line 5.1 were free of symptoms upon inoculation at high whiteflies pressure at a very early stage of plant development (Bonfim et al. 2007). After that this variety was subject to characterization in order to confirm the transgene insert, its stability for at least eight self-pollinated generations and backcrosses with nontransgenic commercial cultivar and absence of siRNA signals on seeds after cooking (Aragão et al. 2013). Although the ISAAA (2016) stated that there are still no GMO common bean areas under cultivation, there are expectations of future commercial initiatives. Figure 5.5 shows transgenic common bean plants, without virus affections even growing under high whitefly pressure. Similarly, the same ISAAA report mentions the advances of INIFAP Mexico in order to obtain transgenic common bean cultivars, possessing the biotech event FMA pdf1.2-INIFAP that confers wide resistance to fungal diseases, very critical in many tropical and subtropical countries.

Because common bean is a strategic crop for food security in many developing countries, much of its production is promoted by international programs, farmers' organizations and governments that in some cases distance themselves from GMO technology, because of the associated controversy. Nonetheless, GMO technology should be considered when available genetic diversity fails to provide novel traits to confront biotic and abiotic stresses. Transgenic technology could provide valuable methods to strengthen breeding programs. Perhaps the perception of GMOs that has led to overregulation will change in future years in light of the discovery of sponta-

Fig. 5.5 Common bean field showing on the left a plot with transgenic variety (AC1-viral gene silenced) showing immunity to BGMV and on the right a commercial variety severely affected (yellowish plants). (Photo: Courtesy of Francisco J.L. Aragão, Embrapa, Brazil, 2018)

neous transgenic organisms prospering in nature (Chiba et al. 2011; Kreuze and Valkonen 2017; Kyndt et al. 2015) and also the safe production of common bean events monitored by Embrapa (Faria et al. 2010; Pinheiro et al. 2014).

5.6 Mutation Breeding

Although common bean holds a considerable amount of genetic diversity, not all the targeted traits can be found by traditional methods during the breeding process. Mutagenesis is a feasible approach considering the advantages over other approaches, such as transgenesis that is overregulated and still causes polemics in some countries. Nevertheless, due to the high costs of equipment and qualified personal, it is important to strengthen international cooperation among laboratories in order to design more robust regional programs.

5.6.1 Conventional Mutagenesis

Among mutation induction methods, the use of ethyl methanesulfonate (EMS, $CH_3SO_3C_2H_5$) has been preferred in common bean with 43.1%, followed by X and gamma rays with 37.2 and 15.6%, respectively. The remaining 4.1% were in minor usage N-nitroso-N-methyl urea ($C_2H_5N_3O$) and ethyleneimine (C_2H_5N) (IAEA

2018). The range of effectiveness for gamma ray treatment (frequency of mutations induced by a unit dose of mutagen) is calculated to be 0.09–0.099 and for EMS treatment 6.86–9.8, and in cases of combination treatment the range is 0.12–0.34, the combination of lower dose of gamma ray and EMS could be more effective (More and Borkar 2016). The use of 40 mM EMS was adequate for generating mutants in common bean, higher concentrations of EMS induced survival rates of less than 10% and lower concentrations reduced the number of mutants (Porch et al. 2009). For gamma rays, 303.17–318.22 Gy are considered proper doses for inducing mutations in common bean varieties (Ulukapi and Ozmen 2017). In a minor way, sodium azide (NaN_3) has been used to induce mutations, proving to be able to broaden the genetic diversity of an improved variety (Chen et al. 2011).

5.6.2 Enhanced Traits and Improved Cultivars

According to the International Atomic Energy Agency (IAEA 2018), 59 mutant common bean varieties were registered (1950–2007), of these 44% were generated in the USA and 20.3% in Canada. In contrast, there were 173 varieties registered for soybean. The main improved attributes of mutant varieties are early maturity, high yield, resistance to different pathogen strains, particular seed colorations and patterns, flower colorations, bush growth habit, high protein content and cooking quality. As the same register shows, most induced mutant varieties also serve as parents for crosses and backcrosses to incorporate novel alleles into the base germplasm.

5.7 Conclusions and Prospects

5.7.1 An Overview of Current Status

It is estimated that the world population will continue to increase in the coming decades and that climate change will make food production more complex, creating food insecurity in many vulnerable countries (Porter et al. 2014). Under these unpredictable scenarios, the common bean is going to play an important role in providing cheap protein and other essential nutrients for human consumption. Common bean breeding activities, from 1930s until today, have been crucial in improving yields, creating resistance to biotic and abiotic stresses and adding nutritional value. Nonetheless, after reviewing breeding history and its achievements, and being aware of current challenges, it is evident that future breeding projects must be strengthened in order to achieve ambitious goals within a short period of time.

The contemporary production and yield gaps among continents and countries suggest that there are opportunities for significant improvements, with subsequent

benefits to farmers, consumers and even the environment. However, current traditional methods of genetic improvement are not sufficient to achieve varieties with high quality in a short period of time. Although many publications indicate advances to better understand molecular processes related to yield formation under stressful conditions, present common bean breeding programs are still based on traditional methods, without incorporating modern methods. Only in a minor cases are some of the modern biotechnology tools being used to incorporate new traits.

International institutes, research centers and NIARs have joined forces to develop regional breeding projects that have achieved important results. However, as most NIARs receive nursery stock of advanced genetic material for evaluation and validation, the development of advanced technical skills among breeders and technicians in the target countries is limited, reducing opportunities for breeding projects relying on local germplasm and expertise. In this sense, the participatory breeding approach has risen in importance in the last decade, providing significant achievements in many countries, but facing limitations in terms of seed systems. Nevertheless, there exist a considerable number of new varieties with high yield potential (Appendix II); but, the use of high-quality seeds and adoption of proper sowing and management practices remains low, reducing the impact of genetic improvement on common bean production.

5.7.2 Current Research Initiatives to Combat Global Climate Change

Section 5.1.2 discussed predicted climate change scenarios based on different models and how common bean production could be affected. In addition to global initiatives to ameliorate the impact of climate change in a broad sense, carrying out common bean breeding has to take into account variables related to resilience to climate change. Multiple programs and projects led by CIAT in Africa, Asia, Latin America and Caribbean can combat climate change affecting the cultivation of common bean by means of genetic improvement on a world scale. The CIAT program aims to release varieties that are high-yielding, tolerant to drought, heat and low-soil fertility, resistant to pest and diseases, nutritionally improved and with market potential. The websites of CIAT (http://ciat.cgiar.org/what-we-do/breeding-better-crops/beans/) and PABRA (Pan-Africa Research Alliance, http://www.pabra-africa.org/) can be consulted to track upcoming information.

5.7.3 Recommendations of Future Research and Utilization

It is very important to recognize that there is great genetic richness among *Phaseolus* species conserved in situ and ex situ, which has not been screened for novel traits. In this respect, and considering the climatic events of the last decade, in situ common bean genetic resources represent a valuable source of novel alleles present in populations prospering under adverse biotic and abiotic conditions. Common bean breeding programs should adopt modern biotechnology tools to conduct germplasm screening and to assist in the selection process. Albeit this approach was not possible before, because of high costs, today new advances have reduced costs significantly (Barabaschi et al. 2016), opening new possibilities for common bean breeders.

We must be aware that some target variants will not be present in germplasm in natural conditions. Thus, interspecific hybridization and mutagenesis could provide important alleles to be incorporated into breeding lines as well. Likewise, as long as functional genomics is advancing for the common bean, we will better understand the processes and dynamics of the genetic expression in response to biotic and abiotic stresses, being able to propose new ways of counteracting the negative effects of climate change on production. It will be important to continue deepening this understanding. Genetic engineering could help to design novel genotypes with special characteristics in the short term, but overregulation of these technologies and the questionable perceptions of both society in general and the political class, currently limit the extent to which these advantages can be brought to bear on future challenges.

Participatory breeding should be strengthened in the coming years focusing on those cropping systems exposed to climate change effects, adopting modern forms to conduct breeding and at the same time considering the conservation of valuable genetic diversity. It will be necessary to expand the variety ideotype to the whole agroecosystems, aiming for varieties that demand fewer inputs and respond better to ecological management using bio-inputs. Finally, although it is not the responsibility of breeders, in addition to releasing a new variety, it is important to promote the use of high-quality seeds and the adoption of proper practices for crop management in order to positively impact common bean production. In this sense, CSBs seem to be a very innovative option for distributing high-quality seeds, but the quality must be ensured by following proper technical practices. CSBs should also be considered as vital spaces for the transfer of technologies that allow educating farmers about new types of cultivation for each variety and in correspondence with climate change, such as the use of bio-inputs and agroecological management.

Appendices

Appendix I: Research Institutes and Online Resources Available for Common Bean Breeding

Institute	Area of specialization and research activities	Contact information
Bioversity International	Plant genetic resources, conservation of common bean genetic resources in situ and ex situ; support to community seed banks for high-quality seed production; plant breeding using participatory approaches	Via dei Tre Denari, 472/a 00054 Maccarese (Fiumicino), Italy https://www.bioversityinternational.org
Brazilian Agricultural Research Corporation (Embrapa)	Plant breeding, agro-biotechnology, crop management, plant breeding for high yield, resistance to biotic and abiotic stresses; tissue culture; genetic engineering	Rodovia GO-462, Km 12, Fazenda Capivara, Zona Rural Caixa Postal: 179 CEP: 75375-000 – Santo Antônio de Goiás GO, Brasil https://www.embrapa.br/en/arroz-e-feijao/
International Center for Tropical Agriculture (CIAT)	Plant breeding, crop management, seed production, phenotypic and molecular characterization of germplasm; plant breeding for high yield, resistance to biotic and abiotic stresses, high grain iron and zinc concentration; pre-breeding; conservation of genetic resources in situ and ex situ; support to community seed banks for high-quality seed production	Km 17 Recta Calí-Palmira CP 763537 Apartado Aéreo 6713, Calí, Colombia Dr. Stephen Beebe (s.beebe@cgiar.org) http://ciat.cgiar.org/what-we-do/breeding-better-crops/beans/
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)	Plant breeding, agro-biotechnology, crop management, genetic improvement using modern genomics, molecular biology and breeding approaches	Patancheru 502324 Telangana, India Dr. Rajeev K. Vashney (r.k.varshney@cgiar.org) http://www.icrisat.org/research-development/
Michigan State University	Plant breeding, crop management, breeding and genetics for drought tolerance and disease resistance; support to community seed banks for high-quality seed production	220 Trowbridge Rd., East Lansing, MI 48824, USA Dr. James D. Kelly (kellyj@msu.edu), http://www.canr.msu.edu/psm/research
Misión Biológica de Galicia	Crop biodiversity, plant breeding, phenotypic and molecular characterization of germplasm; breeding for resistance to biotic and abiotic stresses	Pazo de Salcedo. Carballeira, 8. Salcedo. 36143 Pontevedra, España Dr. Marta Santalla (msantalla@mbg.csic.es) http://www.mbg.csic.es

(continued)

Institute	Area of specialization and research activities	Contact information
National Institute of Forestry, Agriculture and Livestock Research (INIFAP México)	Plant breeding, plant genetic resources conservation, crop management, plant breeding for resistance to biotic and abiotic stresses; conservation of genetic resources in situ and ex situ; plant breeding using participatory approaches; support to community seed banks for high-quality seed production	Avenida Progreso No. 5, Col. Barrio de Santa Catarina, Delegación Coyoacan C.P. 0401, México, D.F. www.inifap.gob.mx
United States Department of Agriculture / Agricultural Research Service	Plant breeding for high yields, resistance to biotic and abiotic stresses and to improve cooking time and nutritional value of harvested seeds	Jamie L. Whitten Building, Room 302A, 1400 Independence Ave., S.W. Washington DC 20250, USA https://www.ars.usda.gov/office-of-international-research-programs/ftf-grain-legumes/
University of California (Davis)	Crop biodiversity, plant breeding, study of evolutionary factors that affect crop biodiversity, plant factors such as gene flow and gene diversification, environmental correlations with crop biodiversity, and human effects on the maintenance and generation of diversity	1 Shields Ave, Davis, CA 95616, USA Dr. Paul Gepts (plgepts@ucdavis.edu) https://biology.ucdavis.edu/ https://biology.ucdavis.edu/people/paul-gepts
University of Puerto Rico	Plant breeding, seed production, plant breeding for high yield, resistance to biotic and abiotic stresses. High-quality seed production	Universidad de Puerto Rico, Mayagüez, Puerto Rico 00681-9000 Dr. James S. Beaver (james.beaver@upr.edu) http://www.upr.edu/
Zamorano University	Plant breeding, crop management, plant breeding for high yield, high grain iron and zinc concentration and resistance to biotic and abiotic stresses. Research on *Phaseolus*-rhizobia interaction, high quality seed production	PO Box 93, Km 30 road from Tegucigalpa to Danlí, Yeguare Valley, Municipality of San Antonio de Oriente. Francisco Morazán, Honduras Dr. Juan Carlos Rosas (jcrosas@zamorano.edu) https://www.zamorano.edu/

Note: These institutions have the most significant role at a global level. Nonetheless, there are a significant number of NIARs, universities and international institutes that also contribute to common bean breeding activities in the world

Appendix II: Genetic Resources of Common Bean

Most Popular Common Bean Varieties in African Countries, Their Characteristics and Site of Cultivation

Country	Variety name	Characteristics	Site of cultivation
Tanzania	Lyamungu 85	Tolerant to drought and diseases. Yield 2–2.5 mt/ha. Large red/brown Calima type seeds	Northern and Western zone
	Lyamungu 90	Tolerant to drought and diseases. Yield 2–2.7 mt/ha. Large red mottle, Calima type seeds	Northern and Western zone
	Uyole 90	Tolerant to ALS and R. Yield 2–2.5 mt/ha. Medium cream/brown stripe seeds	Southern highlands
	SUA 90	Tolerant to ALS and R. Yield 2–2.5 mt/ha. Small beige seeds	Eastern zone
	Selian 94	Tolerant to A and storage pests. Yield 2–2.5 mt/ha. Medium pink with red spots seeds	Northern and Western zone
	Uyole 94	Tolerant to ALS, R. Yield 2–3 mt/ha. Large cream/dark red seeds	Southern highlands
	Njano-Uyole	Tolerant to ALS and R. Yield 2–3 mt/ha. Medium yellow seeds	Southern highlands, Western and Northern zones
	Uyole 96	Tolerant to R and ALS. Yield 2–2.5 mt/ha. Large dark red kidney seeds	Southern highlands
	JESCA	Drought tolerant, early maturing variety. Yield 2–2.5 mt/ha. Large purple rounded seeds	Northern and Western zone
	Selian 97	Tolerant to ALS and R. Yield 2–2.5 mt/ha. Large dark red kidney seeds	Northern and Western zone
	Uyole 03	Tolerant to A, ALS and HB. Yield 2–2.5 mt/ha. Large sugar/red specked seeds	Southern highlands
	Wanja	Tolerant to drought due to its early maturing nature. Yield 1.5–2 mt/ha. Large khaki seeds	Southern highlands
	Uyole 04	Tolerant to A, ALS and HB. Yield 2.5–3 mt/ha. Medium cream seeds	Southern highlands
	Calima-Uyole	Tolerant to A and ALS. Yield 2–3 mt/ha. Red mottled (Cranberry) medium seed size seeds	Southern highlands, Western and Northern zone
	Cheupe	Tolerant to A, ALS and HB. Yield 4–6.5 mt/ha. Medium white seeds	Northern and Western zone

(continued)

Country	Variety name	Characteristics	Site of cultivation
	Selian 06	Tolerant to A, ALS and HB. Yield 4.6–7.5 mt/ha. Medium purple seeds	Northern and Western zone
Ethiopia	Lehode	Tolerant to foliar diseases	Northeastern
	Loko	Tolerant/Resistant to ALS, HB, A, BCMNV	Western
	Batu	Tolerant/Resistant to ALS and BCMNV	In areas with short season
	Deme	Tolerant/Resistant to ALS and BCMNV	In all bean growing areas
	Kufanzik	Tolerant/Resistant to ALS, HB, A, BCMNV	Eastern (Hararghe highlands)
	Dursitu	Tolerant/Resistant to ALS and BCMNV	Eastern (Hararghe highlands)
	Hawassa Dume	Tolerant/Resistant to ALS, HB, A, BCMV	Southern region (Wolaita, Sidama, Gamu Gofa)
	CRANSCOPE	Tolerant/Resistant to ALS and BCMNV	Central Rift Valley
	ACOS RED	Tolerant/Resistant to ALS and BCMNV	Central Rift Valley and southern region
	GABISA	Resistant to CBB	Western bean growing region
	Chercher	Tolerant/Resistant to ALS, HB, A, BCMV	Eastern (Hararghe highland)
	Haramaya	Tolerant/Resistant to ALS and BCMNV	Eastern (Hararghe highland)
	Chore	Tolerant/Resistant to ALS and BCMNV	Central Rift Valley and Eastern
	Dinkinesh	Tolerant to CBB	All bean growing areas
	Melkadima	Tolerant/Resistant to ALS and BCMV	Southern and Southwest
	Batagonia	Tolerant/Resistant to ALS and BCMNV	Southern
	Anger	Tolerant/Resistant to ALS and BCMNV	Western
	Tibe	Tolerant/Resistant to ALS, HB, A, BCMNV	Western
	Wedo	Tolerant/Resistant to ALS and BCMV	Northwest
	Ibado	Tolerant/Resistant to ALS and BCMNV	Southern
	Omo-95	Tolerant/Resistant to ALS	Southern
	Nasir	Tolerant/Resistant to ALS and BCMV	Across all bean growing regions
	Dimtu	Resistant to BGMV	Across all bean growing regions

(continued)

Country	Variety name	Characteristics	Site of cultivation
	Tabor	Tolerant/Resistant to ALS and BCMNV	Central Rift Valley and Southern
	Zebra	Tolerant/Resistant to ALS, HB, A, BCMNV	Across all bean growing regions
	Gobe Rasha-1	Tolerant/Resistant to ALS and BCMNV	Southern and Southwest
	Red Woliata	Tolerant/Resistant to ALS and BCMNV	Southern
	Awash Melka	Tolerant/Resistant to ALS, HB, A and BCMV	All bean growing regions
	Roba	Tolerant/Resistant to ALS, HB, A and BCMNV	All bean growing regions
	Awash 1	Tolerant/Resistant to ALS, HB, A and BCMNV	All bean growing regions
	Mexican 142	Resistant to ALS	All bean growing regions
Kenya	New Rose Coco	Moderate resistance to R, CBB, ALS, A, BCMV and BCMNV. Yield 1.3–2.3 mt/ha. Large/ calima type seeds	Eastern, Western and Rift valley
	Miezi mbili	Resistance to R, CBB, ALS, A, BCMV and HB. Yield 1.2–2.3 mt/ha. Large seeds	Central, and Rift valley
	Kenya Early	Moderate resistance to R, CBB, ALS, A and BCMV. Yield 1.1–2.2 mt/ha. Large seeds	Eastern, Nyanza, Central, Western and Rift valley
	Kenya Red Kidney	Moderately resistance to R, CBB, ALS, A, BCMV and BCMNV. Yield 1.1–2.8 mt/ha. Large seeds	Eastern, Nyanza, Central, Western
	Kenya Wonder	Moderate resistance to HB, CBB, ALS, A and BCMV. Yield 1.1–2 mt/ha. Large seeds	Eastern, Nyanza, Central, Western and Rift valley
	Kenya Sugar Bean	Moderate resistance to HB, CBB, ALS, A and BCMV. Yield 1.1–1.8. mt/ha. Large seeds	Eastern, Nyanza, Central, Western and Rift valley
	Tasha	Resistant to ALS, A and RR. Yield 1.1–2.1. mt/ha. Large/calima type seeds	Eastern and Rift valley
	Kenya Afya	High grain iron and zinc concentration, medium and brownish yellow seeds. Yield 2.2–3.2 mt/ha. Calima type seeds	Eastern, Nyanza, Central, Western and Rift valley
	Kenya Majano	High grain iron and zinc concentration. Yield 2.2–3 mt/ha. Medium and yellow seeds	Eastern, Nyanza, Central, Western and Rift valley
	Kenya Madini	High grain iron and zinc concentration. Yield 2.2–2.5 mt/ha. Calima type seeds	Eastern, Nyanza, Central, Western and Rift valley

(continued)

Country	Variety name	Characteristics	Site of cultivation
	Kenya mavuno	Resistant to A and CBB. Yield 2–3 mt/ha. Medium/Calima type seeds	Eastern, Nyanza and Central,
	Kenya Safi	Resistant to A. Yield 1.2–1.5 mt/ha. Medium grains/Calima type seeds	Eastern, Nyanza, Central, Western and Rift valley
	Mwitemania	Drought tolerant. Yield 1.2–1.5 mt/ha. Medium size/pinto seeds	Eastern, Nyanza, Central, and Rift valley
	Katheka (KATB 1)	Early maturity, heat and drought tolerant, cooks fast. Yield 1.2–1.5 mt/ha. Medium round yellow seeds	Nyanza, Central, Western and Rift valley
	KATB 9	Tolerant to heat, high yielding, drought tolerant, early maturing, cooks fast. Yield 1–1.8 mt/ha. Medium round red seeds	Eastern, Nyanza, Central, Western and Rift valley
Malawi	Namajengo	High yielding. Yield 2.5 mt/ha	Livingstonia, Viphya, Dedza
	Kanzama	High yielding and wide adaptation. Yield 2.5 mt/ha	Chitipa, Livingstonia, Viphya
	Kalima	Tolerant to ALS and A. 2 mt/ha. Large seeds	Chitipa, Livingstonia, Viphya, Dedza
	Bunda 3	Resistant to BCMV. Yield 2 mt/ha	Lake Basin, Phalombe
	Kambidzi	High yielding, tolerant to ALS. Yield 2.5 mt/ha	Lake Basin, Phalombe
	Nagaga	Tolerant to low soil fertility, resistant to BCMV. Yield 2 mt/ha	Mzimba, Lilongwe, Dowa, Nmawera, Shire
	Kabalabala	Tolerant to ALS and CBW. Yield 2.5 mt/ha	Lake Basin, Phalombe
	NUA 59	Early maturing, high grain iron and zinc concentration. Yield 1.7 mt/ha	Mzimba, Lilongwe, Dowa, Nmawera, Shire
	Iris	Drought tolerant, early maturing. Yield 3.5 mt/ha. Carioca type seeds	Guruve, Gokwe south and Nyanga
	Cardinal	Wide adaptation. Yield 4 mt/ha. Calima type seeds	Kwekwe, Marondera, Chipinge and Lupane
	Speckled Ice	Wide adaptation. Yield 3.5 mt/ha. Sugar type seeds	Chimanimani, Shrugwi, Binga and Chirumanzu
	NUA 45	Good taste, high grain iron and zinc concentration, quick to cook. Yield 2.4 mt/ha. Calima type seeds	Guruve, Gokwe south and Nyanga

(continued)

Country	Variety name	Characteristics	Site of cultivation
	Gloria	Attractive seed color (local market). Yield 2.4 mt/ha	Chimanimani, Shrugwi, Binga and Chirumanzu
	Bounty	Yield 2 mt/ha. Sugar type seeds	Chimanimani, Shrugwi, Binga and Chirumanzu
	PAN148	Widely adapted, resistant to BCMV. Yield 2.1 mt/ha. Sugar type seeds	Kwekwe, Marondera, Chipinge and Lupane
	PAN127	Moderately tolerant to rust and resistant to BCMV. Yield 1.6 mt/ha. Sugar type seeds	Kwekwe, Marondera, Chipinge and Lupane
Uganda	NABE 1	Tolerant to ALS, A and BCMV. Medium/large/sugar/red mottled/yellow seeds	Western and Eastern Tall grass
	Kanyebwa	Tolerant to ALS, A and BCMV. Medium/large/sugar/red mottled/Yellow seeds	Western and Eastern Tall grass
	Nambale	Tolerant to ALS, A and BCMV. Medium/large/sugar/red mottled/Yellow seeds	Western and Eastern Tall grass
	NABE 4	Tolerant to ALS, A and BCMV. Medium/large/sugar/red mottled/Yellow seeds	Western and Eastern Tall grass
	K132, Kanyebwa, Otawa, NABE13, NABE 12C and Kamwanyi	Tolerant to ALS, CBW, wide adaptation. Sugar, medium to large red mottled, small to medium red and brown seeds	Eastern tall grass and Mt. Elgon regions
Burundi	Magorori	Tolerant to BCMV, A, BR and R; intermediate reaction to ALS. Yield 1.2–2 mt/ha. Medium grains/calima seeds	All high-altitude areas in Burundi
	Murengeti	Tolerant to ALS, BCMV, R, BR and A; intermediate reaction to HB. Yield 1.5–2 mt/ha. Large grains/kablanket seeds	All high-altitude areas in Burundi
	Kinure	Tolerant to ALS, BCMV, A, BR and R. Yield 1.5–2 mt/ha. Medium/purple seeds	All high-altitude areas in Burundi
	Mbunduguru	Tolerant to BCMV, A and BR; resistant to ALS; Intermediate reaction to R. Yield 1–1.3 mt/ha. Medium round yellow seeds	Low to medium altitudes
	Inakayoba	Tolerant to BCMV, ALS and R; resistant to A and BR.	Low to medium altitudes

(continued)

Country	Variety name	Characteristics	Site of cultivation
	Inamunihire	Tolerant to A, ALS, BCMV; intermediate reaction to BR. Yield 1.2–2 mt/ha. Large/yellow seeds	Medium altitudes
	Mubogora	Tolerant to A, ALS, BCMV. Yield 1–1.5 t/ha. Large/red kidney seeds	Medium to high altitudes
	Bishaza	Resistant to ALS; tolerant to BCMV and CBB; intermediate reaction to A. Yield 1 mt/ha. Medium/Sugar seeds	Medium to high altitudes
	Bisera	Tolerant to BCMV, ALS, A, BR and RR. Yield 1–1.5 mt/ha. Large/red mottled seeds	Medium altitudes

Sources: Monyo Emmanuel and Laxmipathi Gowda (2014); Mukankusi et al. (2018); Katungi et al. (2017). Papias H. Binagwa is acknowledged for contributing to this table design
Key: *A* Anthracnose, *ALS* Angular Leaf Spot, *BCMNV* Bean Common Mosaic Necrosis Virus, *BCMV* Bean Common Mosaic Virus, *BGMV* Bean Golden Mosaic Virus, *BR* Black Root, *CBB* Common Bacterial Blight, *CBW* Common Bacterial Wilt, *HB* Halo Blight, *R* Rust, *RR* Root Rot

Most Popular Common Bean Varieties in Central American Countries, Their Characteristics and Site of Cultivation

Country	Variety name	Characteristics	Site of cultivation
Costa Rica	Suru	Days to harvest 74–80 days. Yield 1.9 mt/ha. 100-SW of 22 g. White seeds	Whole country, recommended in Brunca region
	Tonjibe	Days to harvest 75–80 days. Resistant to BCMV. Yield 1.5 mt/ha. 100-SW of 23 g. Red seeds	Whole country, recommended in Brunca region
	Chánguena	Days to harvest average 75 days. Resistant to BCMV. Yield 2.3 mt/ha. 100-SW of 21 g. Red seeds	Whole country, recommended in Central region
	Curré	Days to harvest 74–79 days. Resistant to BCMV. Yield 1.8 mt/ha. 100-SW of 21.5 g. Red seeds	Whole country, recommended in Central region
	Gibre	Days to harvest 65–70 days. Resistant to BCMV. Yield potential until 2.5 mt/ha. Red seeds	Whole country, recommended in Central region
	Telire	Days to harvest 72–80 days. Resistant to BCMV and BGMV. Yield 1.8 mt/ha. 100-SW of 23 g. Small red seeds	Whole country, recommended in Brunca region

(continued)

Country	Variety name	Characteristics	Site of cultivation
	Cabécar	Days to harvest 72–75 days. Resistant to BCMV and BGMV. Yield 1.9 mt/ha. 100-SW of 24 g. Small red seeds	Whole country, recommended in north Huetar region
	UCR 55	Days to harvest between 80 and 104 days. Yield 2.3 mt/ha. Black seeds	Whole country, recommended in sites above 840 m under sea level
	Bríbrí	Days to harvest 76–80 days. Resistant to BCMV. Yield 1.7 mt/ha. 100-SW of 18–20 g. Small red seeds	Whole country, recommended in Chorotega region
El Salvador	CENTA Ferromás	Resistant to BCMV and BGYMV. Yield 1.5 mt/ha. High grain iron and zinc concentration. Small red seeds	Most regions
	CENTA Nahuat	Resistant to BCMV and BGYMV. Yield 1.6 mt/ha. Small red seeds	Most regions
	CENTA CPC	Resistant to BCMV and BGYMV. Tolerant to heat and drought. Yield 1.4 mt/ha. Small red seeds	Most regions
	CENTA Pipil	Resistant to BCMV and BGMV. Tolerant to heat and drought. Small red (semi-dark) seeds	Most regions
	CENTA San Andrés	Resistant to BCMV and BGMV. Tolerant to heat and drought. Small red (light) seeds	Most regions
	CENTA 2000	Resistant to BCMV and BGMV. Tolerant to R. Tolerant to heat and drought. Small red (semi-dark) seeds	Most regions
Guatemala	ICTA Chortí	Days to harvest in average 78 days. Tolerant to R, BGMV, ALS and drought. High grain iron and zinc concentration. Yield 1.9 mt/ha. Opaque black seeds	Regions close to the conditions of Jutiapa, Jalapa and Chiquimula
	ICTA Peten	Days to harvest in average of 78 days. Tolerant to R and BGMV. High grain iron concentration. Yield 2.2 mt/ha. Black seeds	Regions close to the conditions of Peten
	ICTA Sayaxche	Days to harvest in average of 88 days. Tolerant to R and BGMV. Yield 2.5 mt/ha. Black seeds	Regions close to the conditions of Peten
	ICTA Superchiva	Days to harvest 120–135 days. High grain iron and zinc concentration. Tolerant to fungus diseases. Yield 1.6 mt/ha. Black seeds	Highland regions
	Hunapú	Days to harvest 120–135 days. Purple pods, Tolerant to R. Yield 1.9 mt/ha. Black seeds	Central and Western Altiplano region
	Altanse	Days to harvest 120–135 days. White pods, Tolerant to R. Yield 1.9 mt/ha. Black seeds	Central and Western Altiplano region
	Texel	Days to harvest 100–110 days. Yield 0.9 mt/ha. Black seeds	Central and Western Altiplano region

(continued)

Country	Variety name	Characteristics	Site of cultivation
Honduras	Honduras Nutritivo	Resistant to BCMV and tolerant to BGYMV. Intermediate tolerance to CBB and R. High grain iron concentration. Small red seeds	Most regions
	Azavache 40	Days to harvest 76–80 days. Resistant to BCMV and BCMNV. Intermediate tolerance to BGYMV, CBB, WB and R. Yield 2.5 mt/ha. Black seeds	Most regions
	Lenca Precoz	Days to harvest 60–70 days. Resistant to BCMV, BCMNV and BGYMV. Tolerant to CBB, WB and R. Yield 2.2 mt/ha. Small black seeds	Most regions
	Cardenal	Days to harvest 65–70 days. Resistant to BCMV and BGYMV. Tolerant to WB and R. Yield 1.8 mt/ha. Small red seeds	Most regions
	Deorho	Days to harvest 70–80 days. Resistant to BCMV and BGYMV. Tolerant to ALS, WB, R, drought, heat and low soil fertility. Yield 2 mt/ha. Small red seeds	Most regions
	Paraisito Mejorado 2	Days to harvest 70–75 days. Resistant to BCMV. Intermediate tolerant to BGYMV, CBB and R. Yield 1.7 mt/ha. Small light-red seeds	Most regions
	Tío Canela 75	Days to harvest 70–80 days. Resistant to BCMV and BGYMV Intermediate tolerance to A and R. Yield 1.7 mt/ha. Small red seeds	Most regions
	Amadeus 77	Days to harvest 70–75 days. Resistant to BCMV and BGYMV. Tolerant to drought. Yield 1.7 mt/ha. Small red seeds	Most regions
	Carrizalito	Days to harvest 70–75 days. Resistant to BCMV and BGYMV. Tolerant to drought. Yield 2.3 mt/ha. Small red seeds	Most regions
Nicaragua	INTA Fuerte Sequía	Days to harvest 72–75 days. Resistant to BCMV and BGYMV. Tolerant to drought and heat. Yield 1.6 mt/ha. Dark red seeds	Most regions
	INTA Precoz	Days to harvest 68–70 days. Resistant to BCMV and BGYMV. Tolerant to drought. Yield 1.3 mt/ha. Small red seeds	Most regions
	INTA Rojo	Days to harvest 75–78 days. Resistant to BCMV and BGMV. Yield 1.6 mt/ha. Light red seeds	Most regions
	INTA Cárdenas	Days to harvest 78–80 days. Resistant to BCMV and BGMV. Yield 1.6 mt/ha. Black seeds	Most regions
	INTA Ferroso	Days to harvest 72–74 days. Resistant to BCMV and BGMV. High grain iron concentration. Yield 1.2 mt/ha. Small red seeds	Most regions

(continued)

Country	Variety name	Characteristics	Site of cultivation
	INTA Nutritivo	Days to harvest 68–72 days. Resistant to BCMV. Yield 1.6 mt/ha. High grain iron concentration. Red (light) seeds	Most regions
	DOR364	Days to harvest 80–85 days. Resistant to BCMV and BGMV. Yield 2.3 mt/ha. Deep dark red seeds	Most regions

Sources: Araya and Hernández (2007); CENTA (2018); DICTA (2018); ICTA (2018); INTA (2018); INTA (2013); Reyes (2012)
Key: *100-SW* average 100-seeds weight, *A* Anthracnose, *ALS* Angular Leaf Spot, *BCMNV* Bean Common Mosaic Necrosis Virus, *BCMV* Bean Common Mosaic Virus, *BGMV* Bean Golden Mosaic Virus, *BGYMV* Bean Golden Yellow Mosaic Virus, *CBB* Common Bacterial Blight, *R* Rust, *WB* Web Blight

Most Popular Common Bean Varieties in South America, Their Characteristics and Site of Cultivation

Country	Variety name	Characteristics	Site of cultivation
Brazil	BRS Ametista	Tolerant to A, CBB and R. Moderate resistance to *Fusarium* wilt. 100-SW of 30 g	East and central regions
	BRS Notável	Resistant to CBB and moderately resistant to A, R, *Fusarium* wilt and *Curtobacterium*. 100-SW of 26 g	East and central regions
	BRSMG Madreperola	Moderate potential resistance to A and ALS. 100-SW of 24.5 g	South-east regions
	BRS Estilo	Adapted to mechanical harvest. Moderately resistant to A and R. 100-SW of 26 g	South and central regions
	BRSMG Realce	High productive potential and excellent culinary properties. Tolerant to A, CBB, R, *Fusarium* wilt and *Curtobacterium*. 100-SW of 43 g	South-east regions
	BRS Radiante	Good culinary quality. Tolerant to A, R, ALS, *Fusarium* wilt and *Curtobacterium*. 100-SW of 44 g	South-east and central regions
	BRS Agreste	Adapted to direct mechanized harvesting. Moderate resistant to A and *Fusarium* wilt. 100-SW of 25 g	East regions
	BRS Vereda	Uniform coloring and excellent culinary properties. Moderately resistant to A, ALS and *Fusarium* wilt. 100-SW of 26 g	South regions
	BRS Pitanga	Excellent culinary properties. Moderate resistance to A, R, ALS and *Fusarium* wilt. 100-SW of 20 g. Red seeds.	West and central regions
	BRS Executivo	It is an option for producers interested in *Sugar Bean* type beans. 100-SW of 76 g	South regions

(continued)

Country	Variety name	Characteristics	Site of cultivation
	BRS Embaixador	Moderate resistance to A and *Fusarium* wilt. Grains favored for the national market, providing price advantages and with potential for export. 100-SW of 63 g. Red seeds	South regions
	Jalo Precoce	Early maturing and tolerant to CBB, R and *Fusarium* wilt. 100-SW of 35 g. Cream seeds	South-east and central regions
	BRS Esplendor	Adapted to direct mechanical harvesting. Resistant to CBB and tolerant to A, R and *Fusarium* wilt and *Curtobacterium*. 100-SW of 22 g. Black seeds	South-east and central regions
	BRS Campeiro	Excellent culinary qualities. Adapted to direct mechanized harvest. Tolerant to A, R and *Fusarium* wilt. 100-SW of 25 g. Black seeds	South-east and central regions

Source: Embrapa (2013)

Note: Due to the special condition explained in Sect. 5.2.2, this appendix contains information about only particular varieties from some African countries, Brazil and Central America illustrating the high genetic diversity available for cultivation and breeding. Furthermore, there are a considerable number of varieties from participatory breeding and thousands of landraces and old cultivars with significant relevance to the food security in the developing world

Key: *A* Anthracnose, *ALS* Angular Leaf Spot, *CBB* Common Bacterial Blight, *R* Rust, *100-SW* Average 100-seeds weight

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Chapter 6
Cowpea [*Vigna unguiculata* (L.) Walp.] Breeding

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Abstract Cowpea, *Vigna unguiculata* (L.) Walp., is an important grain legume grown and consumed not only in the dry savannah areas of Sub-Saharan Africa but also in many other tropical and subtropical regions. It provides income, food and nutrition security to millions of people. Several studies have led to a better understanding of the taxonomy of cowpea and its wild relatives. The species diversity, distribution and evolution of cowpea have been intensively explored. The crop is mainly cultivated in intercropping system where its low plant population does not allow the full expression of the yield potential of the cultivars being grown. Considerable challenges affect the production of this crop despite its comparatively better adaptation to harsh environments. The available genetic resources maintained in the different gene banks are being used for the improvement of cowpea. Germplasm diversity and cultivars characterization were conducted in different studies. Sources of resistance/tolerance to key biotic and abiotic stresses are being identified and introgressed genes involved in new breeding lines are being developed. Improvement strategies were developed to address the major constraints to production while also taking consumer preferences into consideration. Breeding approaches of self-pollinated crops were used in the breeding programs. Application of biotechnology has been suggested to address intractable problems. Considerable effort has been made to genetically transform cowpea. Recent development of genomic resources should support the implementation of molecular breeding to complement conventional breeding and to enhance genetic gain. Key elements needed for successful application of molecular breeding tools include the availability of a high-throughput genotyping platform, high-quality consensus genetic maps,

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improved phenotyping capability and identification of markers closely linked to target traits. Progress is being recorded in many of these areas which should allow the development of modern breeding programs that will result in effective and efficient development of improved resilient cowpea cultivars.

Keywords Conventional breeding · Cowpea · Genetic resources · Genomics · Modern Breeding · *Vigna unguiculata*

6.1 Introduction

Cultivated cowpea, also called black-eyed pea (*Vigna unguiculata* (L.) Walp.) is a commonly grown and consumed grain legume, which is very well adapted to the dry savannah areas of Sub-Saharan Africa (SSA). It belongs to the subfamily Faboideae, tribe Phaseoleae, subtribe Phaseolineae, genus *Vigna* section *Catiang*. The genus *Vigna* is divided into numerous species with numbers ranging from about 150 (Verdcourt 1970) to 184 (Phillips 1951) and 7 sections (Verdcourt 1970). The classification of *Vigna* species has remained generally inconclusive for quite some time. Several researchers have made efforts to resolve the *Vigna* species classification issue (Maréchal et al. 1978; Padulosi 1993; Pasquet 1993; Pienaar 1992). In recent reports, Pasquet and Padulosi (2012) synthesized the work of Vaillancourt et al. (1993) and Delgado-Salinas et al. (2011) on the taxonomic boundaries of the genus *Vigna* thus suggesting the existence of five subgenera as follows:

(a) *Lasiospron*;
(b) *Vigna* (now including yellow and blue flowered species such as *V. subterranean*);
(c) *Haydonia*;
(d) *Ceratotropis* (Asian subgenus);
(e) *Plectotropis* (including section *Catiang* with two species *V. unguiculata* {cowpea} and its sister species *V. schlechteri* Harms previously referred to as *V. nervosa* Markotter).

De Leonardis et al. (1993) reported that members of section *Catiang* have canoe-shaped keel pointed like a beak at the top and pollen grains have reticulate surfaces. However, the cultivated cowpea along with its cross compatible wild relatives are grouped in *Vigna unguiculata* subspecies *unguiculata*.

Pasquet and Padulosi (2012) concluded that the position of *Vigna* within Phaseolinae is established. Cultivated cowpea, *Vigna unguiculata* subspecies *unguiculata* is divided into four cultivar groups as follows: *Unguiculata*, *Sesquipedalis*, *Biflora* and *Textilis*. The cultivar group *Unguiculata* is made up of the cowpea generally grown for the protein-rich grains and fodder for livestock while *Sesquipedalis* comprises the yard-long-bean which is most commonly grown in Asian countries, especially India and China. It is suggested that this cultigroup evolved from cowpea in Asia following selection for long podded types that are

consumed as vegetable – both grains and fleshy pods. The cultigroup *Textilis* has long fibrous peduncles, which are used in northern parts of Nigeria to make rope from the fiber.

The primitive wild relatives of cowpea such as *Vigna unguiculata* ssp. *dekindtiana*, ssp. *stenophylla,* ssp. *tenuis,* ssp. *pubescens* and ssp. *protracta* as well as several cultivars like ssp. *tenuis* var. *tenuis*, var. *oblonga*, var. *parviflora*, var. *ovata* and ssp. *protracta* var. *protracta,* var. *rhomboidea* among others, are most commonly found in southern parts of Africa. These are distributed across from Namibia through Zambia, Botswana, Zimbabwe, Mozambique, Eswatini (Swaziland) and South Africa (Padulosi et al. 1991).

6.1.1 Domestication

The West and Central Africa subregions have been suggested to be the center of origin of cultivated cowpea since it is there that the greatest amount of germplasm is present. The immediate progenitor of cultivated cowpea has been reported to be *Vigna unguiculata* ssp. *dekindtiana* var. *dekindtiana*. It is the most cross compatible with cowpea and seeds of some *dekindtiana* lines though small in size are bigger than those from the wild types found in southern parts of Africa. In a review of previous work that was based on plant morphology, Faris (1965) suggested there was enough evidence to show that cultivated cowpea was domesticated in West or Central Africa. The movement of cowpea to other parts of the world such as Europe, Asia and the Americas is said to have taken different routes. While cowpea movement to Asia especially India was from West Africa through northeastern Africa along with sorghum, which is adapted to similar agro-ecologies as cowpea (Faris 1965; Pant et al. 1982) movement to other parts especially to the USA was through African slaves who took along some seeds (Whit 2007). Movement of cowpea to Europe was through Egypt in North Africa (Ng and Singh 1997). The yard-long-bean, *V. unguiculata* ssp. *unguiculata* cultivar group *Sesquipedalis*, evolved in Asia where it is most commonly found and cultivated for consumption of the fresh green pod with seeds as a vegetable.

6.1.2 Importance

Cowpea is a food and nutrition security crop in different parts of SSA. Farmers and food vendors derive income from cowpea, which also provides fodder for ruminants. Being a legume, cowpea fixes atmospheric nitrogen some of which it uses for its growth and development and leaves some in the soil for the benefit of companion and following crops. The rapid growth rate of cowpea in the field enables the canopy to cover the soil thereby helping to reduce soil erosion. Apart from being a source of food, feed and income cowpea also contributes to the sustainability of the

cropping system and the environment. The grains are processed into several types of dishes for human consumption. The most common dish made of cowpea across several parts of West Africa is *akara* or *kosai*. To make *akara* the cowpea grain is ground into a paste and deep fried in oil in small balls. Cowpea is a known source of dietary protein in many communities where meat is very expensive to purchase. Besides the protein content which can be up to 32% on a dry weight basis (Nielsen et al. 1993) the grains also contain carbohydrates (62% soluble carbohydrates) and according to Boukar et al. (2011) minerals such as iron (33.6–79.5 mg/kg), zinc (22.1–58 mg/kg), phosphorus (3450–6750 mg/kg), calcium (310–1395 mg/kg) magnesium (1515–2500 mg/kg) and potassium (11,400–18,450 mg/kg). Compared with some other food legumes cowpea is rich in amino acids such as lysine, methionine and tryptophan but deficient in sulfurous amino acids. The grains also contain trypsin inhibitor, but the level is about one-half that contained in soybean (Lambot 2002). Cooking, however, inactivates the inhibitor. Cowpea leaves are also consumed as a vegetable in several East African countries especially Kenya and Tanzania. The green leaves contain 29–43% protein on a dry weight basis with younger ones having higher amounts (Nielsen et al. 1997). Because cowpea, when compared to several other crops, is drought tolerant, it promises to be better suited to the dry savannah regions that are already characterized by increased frequency of short raining seasons due to climate change.

Most farmers in the dry savannah areas of SSA keep livestock, which they feed with cowpea haulm that is well appreciated by the farmers because of the appreciable level of protein present. Many farmers derive almost the same amount of income from sales of grain as from fodder harvested from their fields. Breeders have in recent times devoted some attention to increasing fodder quality and yield in cowpea because of the economic value of the haulm (Samireddypalle et al. 2017). A number of dual purpose (grain and fodder) cultivars have been selected by breeders to meet the farmers' needs for livestock fodder. The genetic variation present among different cowpea lines in fodder quality and quantity gives breeders opportunities to make progress in selecting for lines with higher levels of these attributes.

6.1.3 Selection and Early Improvements

The history of cowpea cultivar development is recent. Spillman (1911, 1913) in the USA carried out genetic studies in cowpea and reported the inheritance of a number of traits that relate to seed. In India, Roy and Richaria (1948) were the first to report making crosses in cowpea with the aim of generating segregating populations from which selections were made for lines with early maturity and other desirable attributes. Not much activity was reported until the 1960s towards the development of improved cowpea cultivars in Africa where the greatest quantity is produced and consumed. The earliest report of cowpea improvement in Africa was at the Potchefstroom College of Agriculture, South Africa (1948). The cowpea breeding

work initiated at the institute was aimed at developing cultivars that are erect with long peduncles that carry pods above the canopy to enable harvesting with a mower and possessing resistance to leaf spot and nematodes. In SSA, the first report of cowpea breeding work in 1956 was from the Northern Nigeria Regional Department of Agriculture. Concerted breeding efforts were initiated in the early 1970s at the International Institute of Tropical Agriculture with collection and ex-situ conservation of several germplasm lines. The collection efforts resulted in the acquisition of several germplasm lines from different parts of SSA and from outside the region.

Germplasm collection was followed with evaluations for identification of superior genotypes, recombining desirable traits from two or more parents, advancement of segregating populations and selecting those showing desired attributes. Selected lines were later tested across many agro-ecologies (IITA 1972). Those showing superior performance in comparison with existing farmers' cultivars were distributed to interested farmers in various communities. Initial selection efforts were focused on breeding lines with early maturity, disease resistance, high grain yield and white or brown seed coat color. With time however, emphasis was placed on selection of lines with resistance to insect pests, diseases (fungal, bacterial, viral), early to medium maturity, day neutral characteristic, rough seed coat texture and high grain yield. In more recent times, selection has focused on developing breeding lines with resistance to *Striga*, high grain yield, large seed size and dual purpose. It has been difficult to find germplasm lines that show sufficiently high levels of resistance to insect pests such as flower bud thrips, maruca pod borer and a complex of pod-sucking bugs. Hence, cowpea cultivars available to farmers can perform optimally when adequate protection against insect pests is provided. The development and release of cultivars with insect resistance will enable farmers to grow cowpea more profitably, enhance their health as they will no longer need to handle potentially toxic synthetic insecticides and to protect the environment because no chemicals will be released while protecting the crop in the field.

This chapter is devoted to cowpea as an important but *orphan* grain legume crop of SSA. The topics covered include cultivation practices, germplasm biodiversity and conservation, crop improvement using both conventional and new methods such as transgenics and molecular marker-assisted selection.

6.2 Cultivation and Traditional Breeding

Cowpea is grown in different agro-ecological zones of the world. Some key cowpea producing countries are shown in Fig. 6.1. Figures shown for Brazil, Benin, Botswana, Chad, Ghana, Guinea-Conakry, Namibia, Sierra Leone, South Sudan and Togo were obtained through cowpea scientists working in the countries. Its production and utilization depend mostly on the agro-climatic conditions of the production environment and the socioeconomic conditions, ethnic culture and traditions of the people. Different biotic and abiotic stresses adversely affect cowpea production. Cowpea research activities (Appendix I) are being implemented in many cowpea

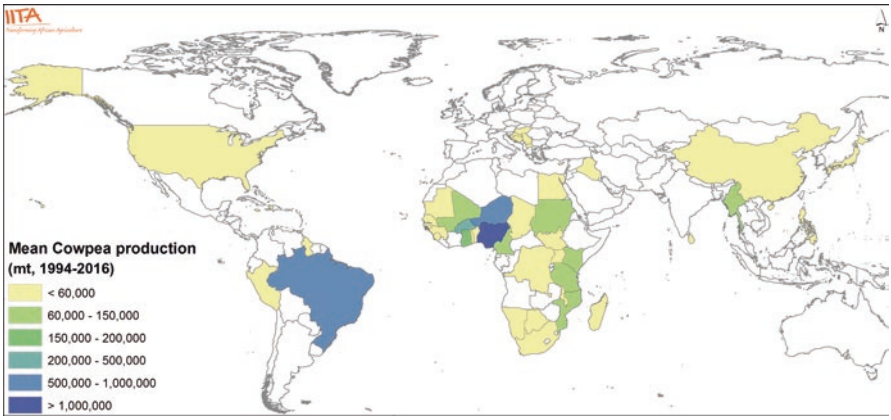

Fig. 6.1 Cowpea production in the world. (Source data: FAOSTAT (2018) and personal communications with scientists (2018))

producing countries to alleviate these constraints. Conventional breeding methods have been used to develop a number of cultivars (Appendix II) and modern breeding tools of molecular markers are now being applied following recent developments of genomic resources.

6.2.1 Current Cultivation Practices

Agronomic practices and the level of inputs (quality seeds, fertilizers, agrochemicals) used for cowpea production differ greatly among farmers. In SSA some farmers still grow local landraces (mostly spreading types) in association mainly with cereals (sorghum, millet, maize). These landraces are characterized by photosensitivity. Cowpea is grown in different types of soils preferably well-drained sandy loams. Generally heavy clay soils are avoided, as the crop is sensitive to waterlogging conditions. Under traditional cultivation, a majority of farmers plant cowpea on flat soil with no ploughing although a few use animal drawn ridgers and still fewer use tractors for land preparation. Weeding of the field is carried out using hand-held hoes. Cowpea yield increases with good land preparation.

Farmers in SSA do not apply fertilizer on cowpea fields. However, the soils are poor with deficiencies in nitrogen, phosphorous and organic matter content. For the crop to produce optimal yield it is recommended to have a starter dose of nitrogen of up to 20 kg/ha. Increase in yield is associated with phosphorus application as single superphosphate at 40 kg P_2O_5/ha (Suzuki et al. 2018). Fertilizer application is done at planting or 7–10 days afterwards.

Typically, cowpea is planted randomly at up to 1.0 m distance between plants and along with cereals. Usually the companion cereal crop is planted first and cowpea later. Where planting is done following straight rows, 0.8050 × 0.40 m is used for local prostrate cultivars while 0.50 × 0.20 m is used for the erect and semi-erect types. Using row planting allows maximizing the crop density and facilitates the application of inputs.

When planted as sole crop or within an organized system, planting is done such that the maturity period of the crop coincides with dry weather. Pod rots cause considerable grain yield loss if harvesting occurs during humid cloudy weather. In addition to the maturity of the cultivars, photosensitivity is also taken into consideration when planning to plant cowpea. Early photo-insensitive cultivars are planted earlier while prostrate photosensitive types may be delayed.

Under intercropping, less than 5 kg seeds are used for planting 1 ha. Generally, these are spreading/prostrate types. In monocropping, 20–25 kg of seeds are required for 1 ha. Most of these are erect and semi-erect types. Until recently seeds were distributed mainly from farmer to farmer. In many cases, grains are purchased and used as seeds. With the efforts of different projects and the deployment of extension agents, farmers organize themselves in groups to produce and sell seeds in their communities. In some countries, seed companies are emerging and they acquire foundation seeds from research institutes and produce certified seeds that are sold in small packs.

In SSA, seeds are usually not treated prior to planting although it is recommended to use seeds free of diseases and insect pests. A good plant stand in the field should lead to high yield. Weed control is one activity that contributes to higher yield in the field. Cowpea may suffer when competing for light, water and nutrients with weeds at its early growth stages. Depending on the region, two to three hand weedings are carried out during the crop life cycle. In some areas, animal tractions are used for weeding and ridging the crop. When pre-emergence herbicides are used, the first weeding can be considerably delayed. Unfortunately, the requisite herbicides are not easily available and most farmers are not aware of their importance. What many farmers are aware of is the use of insecticides to protect their crop. Having one to two targeted insecticide applications is a prerequisite for good grain yield.

6.2.2 Current Agricultural Problems and Challenges

Despite its resilience to drought and low soil fertility, cowpea production is considerably affected by numerous constraints. In addition to biotic and abiotic stresses, there are agronomic practices and socioeconomic challenges that limit significantly the production of this crop in SSA.

6.2.2.1 Biotic Stresses

Insect pests are considered the most limiting factors for cowpea production in most parts of the tropics where appropriate insecticides are lacking or unaffordable by farmers. Insects attack cowpea from the seedling stage to seeds in storage. In addition, different groups of pests infest the cowpea plant at the same time (Jackai and Adalla 1997). At the seedling stage, aphids (*Aphis craccivora* C.L. Koch), beanflies (*Ophiomyia* spp.) leafhoppers (mainly *Empoasca* spp.), foliage beetles (*Ootheca* spp., *Medyrthia* spp. and others), the arctiid defoliator (*Amsacta moloneyi* Druce) and some foliage beetles infest cowpea. During flower bud initiation and flowering time, the most important insect pests are flower bud thrips (*Megalurothrips sjostedti* Trybom) and in some cases maruca (*Maruca vitrata* Fabr.). At podding stage there are *Maruca vitrata*, *Clavigralla* spp., *Acanthomia* spp. and *Riptortus* spp. causing damage to cowpea pods and seeds contained therein. During storage, bruchid (*Callosobruchus maculatus* Fabr.) is the most important insect pest (Boukar et al. 2013, 2015).

Several diseases afflict cowpea and may cause appreciable grain yield loss. The main categories of diseases are bacterial, fungal and viral. Among the bacterial diseases are bacterial blight and bacterial pustule. Major fungal diseases include anthracnose, *Macrophomina*, *Fusarium* wilt, web blight, brown blotch, *Cercospora* leaf spot, *Septoria* leaf spot and scab. Viral diseases recorded in cowpea production areas are cowpea yellow mosaic, cowpea aphid borne mosaic, black-eyed cowpea mosaic, cowpea severe mosaic and southern bean mosaic (Boukar et al. 2013, 2015).

Other biotic constraints are parasitic weeds and nematodes. *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* Benth are main parasitic weeds that are found predominantly in West and Central Africa (WCA) and Eastern and Southern Africa (ESA) respectively. Nematodes such as root knot nematodes induce significant yield losses in susceptible cowpea cultivars.

6.2.2.2 Abiotic Stresses

Drought affects cowpea production adversely despite the ability of the crop to grow under hot weather conditions with little rainfall during the short (55 day) cropping season (Hall and Patel 1985). All the types of drought – seedling stage, reproductive stage and terminal can be experienced by cowpea.

Heat reduces significantly cowpea grain yield when high temperatures (>20 °C) occur late at night. This is because flower production and pollen viability are affected by high temperatures (Hall 2004). This author showed that each 1 °C above a threshold of 16 °C during the night leads to 4–14% reduction in grain yield.

Cowpea production is also reduced due to low soil fertility. In the savanna agroecology, low soil fertility is common due to low organic matter and phosphorous contents. It is reported that soil fertility is more limiting to cowpea grain and fodder production in the Sahelian zone than rainfall and the use of fertilizer can increase water-use efficiency (Penning de Vries and Djiteye 1991).

6.2.3 Improvement Strategies

To alleviate the various cowpea production constraints, improvement programs were established in several countries in Sub-Saharan Africa such as Nigeria, Niger, Senegal, Burkina Faso, Uganda, Kenya and Tanzania with major attention from 1960 onward (Singh and Ntare 1985). During the same period of time, there were substantial efforts in Asia to breed cowpea to suit local cropping systems and consumer tastes. Most of the literature on development of cowpea cultivars comes from India, as well as from other countries such as Bangladesh, Myanmar, China, Indonesia, Nepal, Pakistan, the Philippines, Sri Lanka and Thailand (Mishra et al. 1985). In Latin America, EMBRAPA in Brazil in collaboration with IITA, initiated early cowpea improvement programs. Other countries in this region with some research activities on cowpea include Colombia, Venezuela, Panama, Trinidad, Nicaragua, Jamaica and Guyana. Cowpea is grown mostly in all of the southern USA with extensive dry seed industries in both California and Texas. Cowpea breeding and evaluation programs have existed in the US since the latter part of the nineteenth century. Some of the main cowpea research institutions in this early period included the Arkansas Agricultural Research Station. Around 1980, cowpea research was being conducted at 28 different locations in the USA. Eight institutions, namely, Auburn University, Clemson University, Louisiana State University, Mississippi State University, Texas A&M University, University of Arkansas, University of California and the University of Georgia and the Agricultural Research Service of USDA had ongoing research programs with clearly identifiable cowpea breeding objectives (Fery 1985).

Early cowpea breeding efforts targeted a better understanding of the botany, morphology, physiology and production constraints. Later high yield potential and seed quality were considered as main objectives of the breeding programs. Responses to day length, crop maturity and crop position within the different cropping systems were also considered in the development of improved cowpea cultivars. Major steps taken in the development of better cowpea cultivars in the 1970s were germplasm collection, evaluation and maintenance and breeding for disease resistance. Subsequently, emphasis focused on breeding for insect resistance, early maturity and improved plant types with desired grain quality (Singh and Ntare 1985). Searches for sources of resistance to different diseases and insect pests were also initiated (Singh et al. 1983) followed by an intensive hybridization program to incorporate these key traits in improved breeding lines. In the 1980s, breeding efforts focused on seed-type preferences in the different regions and assessments of damages caused by insect pests along with multiple diseases. It was the aim of breeders at this earlier time to develop single lines with resistance to all the major cowpea diseases in the humid and subhumid tropics. Hence all segregating populations were evaluated for resistance to several diseases from F2 to F6 both under natural or supplemented infestation in the field and glasshouse. As for resistance to insect pests, efforts were devoted to aphids, thrips and bruchids. To this end germplasm lines were evaluated in the field and screenhouse for detection of resistant

lines. A germplasm line TVu3000 was found to be resistant to aphids and the dominant gene controlling the trait was transferred to several improved cowpea breeding lines. Similarly, line TVu 2027 and a land race from Ghana Sanzi were found to show tolerance to storage weevils and flower bud thrips, respectively. These tolerance genes have been incorporated into a number of improved breeding lines. As with insect resistance, germplasm lines were also evaluated for resistance to diseases and those that showed the desired levels of resistance were used as parents in crosses from which segregating generations were assessed for their reactions to the diseases. For example, a local land race, Dan Ila was detected as being resistant to bacterial blight. The resistance gene has been transferred to many improved breeding lines. During the same period, a systematic program was initiated to develop extra-early cowpea cultivars that fit into multiple-cropping systems (Singh and Ntare 1985).

In the 1990s, the IITA breeding program focused on the development of high yielding bush-type vegetative cowpea cultivars with different maturity periods (extra-early, early, medium, late), photoperiod sensitive and insensitive grain types and adaptation to different cropping systems (sole crop, intercrop) (Singh et al. 1997).

In the early 2000s, cowpea cultivars with preferred seed types resistant to biotic (diseases, insect pests, parasitic weeds) and abiotic (heat, drought) and adapted to both sole cropping and intercropping were developed (Singh et al. 2002). From 2010 and thereabouts these efforts were sustained with the aim of developing high yielding widely adapted and stable cowpea cultivars (Fig. 6.2) with resistance to major production constraints and with acceptable seed types (size, texture, color, protein and mineral content, improved cooking properties). The implementation of the Collaborative Research Program of CGIAR on Grain Legumes offered the opportunity to strengthen the development of cultivars with tolerance to drought and low-soil phosphorous and resistance to insect pests. Recent improvement strategies are building on the use of modern approaches in cowpea breeding (Ehlers et al. 2012).

6.2.4 Traditional Breeding Methodologies and Limitations

Being self-pollinated, cowpea cultivar development has benefited from the breeding methodologies applicable to this group of crops. Many cultivars were obtained at the beginning of the breeding programs using mass selection and pure line approaches. Landraces collected from farm fields were evaluated and single plants found to be of good performance were selected. In the case of mass selection, seeds from these plants were bulked and grown to produce improved populations where further selections could be repeated several times. For pure-line breeding, the seeds from each selected plant were sown as progeny rows. Seeds of the best rows were evaluated in replicated yield trials and superior lines selected to constitute new improved cultivars.

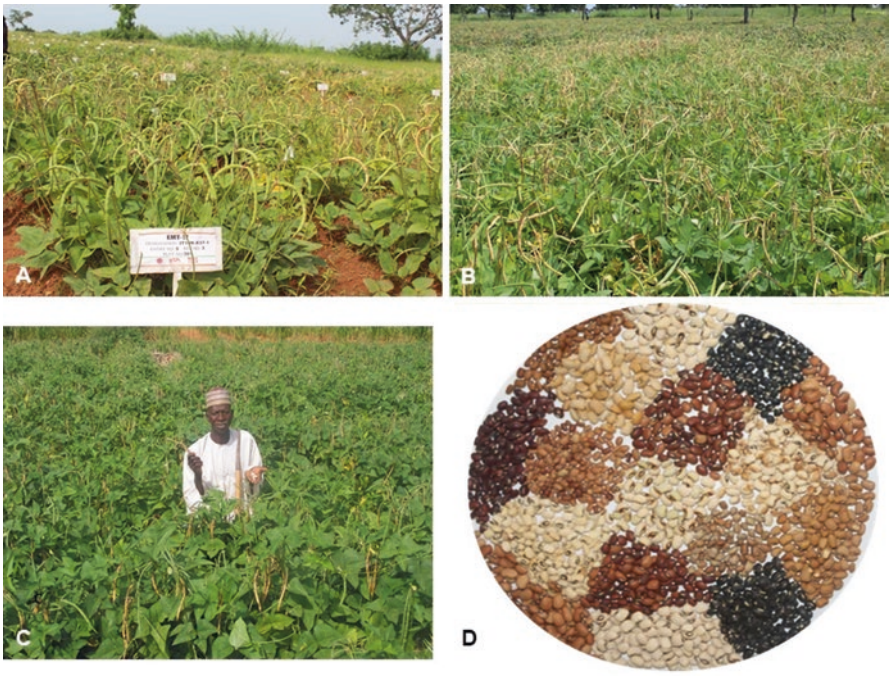

Fig. 6.2 Fields of improved cowpea cultivars. (**a**) evaluation of advanced lines in Tamale, Ghana, (**b**) Seed multiplication in Saria, Burkina Faso, (**c**) Farm field in Kano, Nigeria, (**d**) Seed diversity in cowpea breeding program, IITA, Kano, Nigeria

Because of the limited genetic variability associated with these two breeding approaches, segregating populations were produced from single or multiple crosses between two or more lines. Depending on the aim of the breeding program, the segregating populations were handled in a number of ways. The pedigree method of breeding is used largely in many cowpea breeding programs. This method has proved suitable for the short-term objective of developing cultivars with new combinations of horticultural characteristics and disease resistance (Fery 1985). Single seed descent has also been used especially for rapid generation of recombinant inbred lines for linkage mapping and QTL identification. Mehta and Zaveri (1997) have reported that single seed descent develops better progenies for yield and yield components than single plant selection. Another method that is commonly used in cowpea breeding programs is the backcross breeding method which has proved to be useful for transferring single resistance genes for specific production constraints into cowpea lines that have good yield performance or are preferred by farmers, but susceptible to or lacking this particular trait. To reduce the time and efforts for record keeping associated with pedigree method, breeders have also used bulk population method. In this method of breeding plants in the segregating populations are harvested in bulk through several generations under natural or artificial conditions.

The different combinations or modifications of the breeding methods mentioned above are being used across cowpea cultivar development programs. Depending on the specific characteristics of the parents that were crossed, the traits of focus in the program, and the targeted environments (off-season, screening facilities, presence or absence of inoculum), IITA often performs a combination of these conventional breeding methods. Fery (1985) reported that a combination of backcross-pedigree breeding method has been used in some programs to transfer desired traits from relatively unadapted genetic backgrounds into well-adapted commercial cultivars. Under this combination of methods only one, two or three backcrosses are conducted while the remainder of the breeding is handled through pedigree procedures.

Despite the progress achieved through conventional breeding methods, there are certain limitations associated with them. Sources of resistance to key production constraints such as insect pests, mainly the pod borer and pod sucking bugs, show low levels of expression in cowpea germplasm lines and cultivars. Unfortunately, a wild cowpea relative *Vigna vexillata* (L.) A. Rich which has good sources of resistance genes to these pests is not cross compatible with the cultivated lines. This has prevented the transfer of the resistance genes into cultivated cowpea. In addition, the traditional cowpea breeding approaches require up to a decade or more to develop improved cultivars largely due to the need to employ sequential and repeated phenotypic evaluations and performance trials (Ehlers et al. 2012). These authors pointed out that in many cases, complex, specialized conditions, techniques and skills are needed to assess phenotypes for selection.

6.2.5 Role of Biotechnology

With the advent of recent advances in biotechnology and genomics (see Sects. 6.4 and 6.5, respectively, of this chapter), cowpea genetic improvement can be made more efficient. With the successful genetic transformation of cowpea and stable transmission of the transgene to progeny according to the Mendelian law of inheritance (Popelka et al. 2006), it is now possible to develop transgenic Bt cowpea cultivars with resistance to the pod borer *Maruca vitrata*. Some modifications have been made to improve the genetic transformation systems, which have led to the development of new transgenic cowpea with resistance to bruchids and caterpillars (Higgins et al. 2012).

6.3 Diversity and Conservation of Germplasm

Major cowpea germplasm collections are conserved at the International Institute of Tropical Agriculture, Ibadan, Nigeria with 15,872 accessions from 90 countries, at Griffins, Georgia, USA with 7146 accessions from 50 countries and at Riverside,

California, USA with 4876 accessions from 45 countries. These gene banks represent the largest repertoires of cowpea biodiversity. In addition to cultivated accessions, IITA holds about 1818 accessions of wild relatives. After the Convention on Biological Diversity (1992), IITA's cowpea collections were placed under Article 15 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRF) thus making them available to the international community for research, food and agriculture.

6.3.1 Germplasm Diversity

Cowpea accessions maintained in the gene banks exhibit important phenotypic variations in qualitative traits such as plant type, seed coat color, flower color and quantitative agronomic traits such as yield, maturity or stress tolerance. Although most of the cultivated cowpea cultivars are erect to semi-erect, accessions with prostrate or climbing growth habits. Pods can be coiled, round, crescent or linear. Peduncles range from <5 cm to >50 cm long in the cultivated cowpea species. Porter et al. (1974) described 6 different patterns of pigmentation of flower and pod, 62 eye colors and 42 eye patterns on the seeds. The maturity cycle of the crop varies from 50 days to more than 120 days. Studies of diversity in cowpea germplasm using morphological and physiological traits have been reported in several publications (Egbadzor et al. 2014a; Ehlers and Hall 1996; Fery and Singh 1997; Perrino et al. 1993).

Recent studies describing cowpea germplasm diversity are based on the analysis of molecular markers. All types of molecular markers are used in the diversity analysis in cowpea. Allozymes (Panella and Gepts 1992; Pasquet 1999, 2000; Vaillancourt et al. 1993), seed storage proteins (Fotso et al. 1994; Oppong-Konadu et al. 2005) and chloroplast DNA polymorphisms (Vaillancourt and Weeden 1992) were explored to describe the genetic relationships of some cowpea germplasm. Other markers employed in diversity studies include restriction fragment length polymorphisms (RFLP) (Fatokun et al. 1993), random amplified polymorphic DNA (RAPD) (Ba et al. 2004; Diouf and Hilu 2005; Fall et al. 2003; Mignouna et al. 1998; Nkongolo 2003; Xavier et al. 2005; Zannou et al. 2008), amplified fragment length polymorphisms (AFLP) (Fang et al. 2007), DNA amplification fingerprinting (DAF) (Simon et al. 2007), simple sequence repeats (SSRs) (Asare et al. 2010; Li et al. 2001; Ogunkanmi et al. 2008; Uma et al. 2009; Wang et al. 2008; Xu et al. 2010) and sequence tagged microsatellite sites (STS) (Vir et al. 2009). More recently single nucleotide polymorphism (SNP) markers described as more effective in diversity assessment compared with other markers such as AFLP and SSR (Acquaah 2007; Egbadzor et al. 2014b; Varshney et al. 2007) have been used in cowpea. Huynh et al. (2013) used SNP to study the structure of cowpea landrace and wild relative populations from African and non-African countries. SNPs have also been used in the estimation of genetic diversity and population structure of cowpea (Fatokun et al. 2018; Xiong et al. 2016).

Despite the existence of natural hybrids between wild and cultivated cowpea genotypes, and the wide variation in phenotypic traits among cowpea accessions, a narrow genetic variability is observed in the cultivated gene pool (Ehlers and Hall 1997). In several studies that evaluated genetic variability based on molecular markers, a single domestication event in the cowpea is considered the basis of the narrow genetic variability of the crop (Asare et al. 2010; Ba et al. 2004; Coulibaly et al. 2002; Padulosi and Ng (1993); Pasquet 2000) attributed the low genetic divergence in cowpea to the self-pollinating nature of the crop.

6.3.2 Cultivar Characterization and Phylogeny

Cowpea cultivars exhibit variable features including morphological, agronomic, physiological and molecular expressions. The collection and documentation of these specific characteristics are very valuable for breeding programs. Cultivar characterization adds value to the gene bank and to the breeding programs as the information generated guides the user to request specific cultivars.

The real center of origin for cultivated cowpea still remains hazy. Early reports have indicated Africa as area of domestication of cowpea, given the exclusive presence of wild cowpea relatives (Steele 1976). Faris (1965) using literature and extensive work involving morphological descriptors concluded that West or Central Africa is the center of domestication of cowpea while Coulibaly et al. (2002) using molecular markers presented northeastern Africa as area of early domestication of cowpea. The theory of West African center of origin is more widely accepted (Baudoin and Maréchal 1985; Maréchal 1978; Ng 1995). However, efforts are needed to clarify the dispersal of cultivated cowpea to other regions of the world, the region of first domestication and sub-domestication (Xiong et al. 2016).

Using a collection of cowpea landraces and wild annual cowpeas from both East and West Africa, Huynh et al. (2013) showed that there are two major gene pools for cultivated cowpea in Africa. Landraces from western Africa and eastern Africa form gene pools 1 and 2, respectively. These landraces are similar to the wild relatives available in the same regions. Therefore domestication processes responsible for the existence of the two gene pools occurred differently. These authors pointed out that landraces within Africa presented lower total genetic variation than landraces outside Africa. Xiong et al. (2016) found three well-differentiated genetic populations and admixtures associated with the regions and countries from where the cowpea cultivars were collected (Table 6.1). These authors also concluded that West and East of Africa are the first domestication regions of cowpea while India is a sub-domestication region.

Table 6.1 Regions and countries with same genetic populations and admixtures

Grouping	Regions	Countries
Cluster I	North America, Latin America, Oceania, Central and East Africa, India and South Africa	Asia (Afghanistan, Iran, Pakistan, Turkey, China), West Africa (Cameroon, Niger) and Europe (Hungary) plus the American cultivars
Cluster II	West Africa	South Africa, India and USA
Cluster III	The American, East Asian, Central West Asian, and European cultivars	Latin America (Brazil, Guatemala, Mexico, Paraguay), Southern Africa (Botswana, Mozambique, Zimbabwe), Central East Africa (Kenya) and Oceania (Australia).

6.3.3 Genetic Resources Conservation Approaches

Preventing the loss of agricultural biodiversity is of the highest global priority given the importance of genetic resources in world food security. A framework for the efficient and effective ex-situ conservation of globally important collections of cowpea was initiated in 2010 with key stakeholders through the support of Global Crop Diversity Trust and the leadership of IITA. An extensive survey on cowpea genetic resources conservation and use (collections, facilities, human resources, ongoing research, networks) was conducted in different institutes across various countries. From the data compiled, an international group of experts was constituted to discuss the state of cowpea conservation and use in Africa. A set of recommendations was made. Dumet et al. (2012) summarized these recommendations which are related to safe conservation of each unique *Vigna* accession, its importance and diversity, and the formation of global information portals.

Over 59,000 accessions of cowpea and other *Vigna* spp. (including wild cowpea relatives and other cultivated *Vigna* other than cowpea) are being maintained in ex situ conditions. International seed processing standards need to be followed to ensure safety of the collections. For cowpea and other *Vigna* germplasm, optimal processing includes artificial dehydration (down to 8% on average), germination tests prior to storage, adequate packaging for medium (5 °C) and/or long-term storages (−20 °C) and monitoring viability at regular intervals during storage. Given the difficulties of many countries to comply with these standards, the need for maintaining at least one copy of each unique accession under international standard storage conditions was recommended (Dumet et al. 2012). Germplasm regeneration procedures need to meet standards that insure genetic integrity and health; Dumet et al. (2008) reviewed these standard regeneration guidelines.

6.3.4 Cytogenetics

The genome size of cowpea is relatively small (620 Mb) consisting of 2n = 2x = 22 chromosomes (Arumuganathan and Earle 1991). The chromosomes are described as extremely small. Detailed descriptions of the cowpea karyotype have been reported. Pachytene bivalents, cells in mitotic prometaphase and in metaphase, were used in the development of karyotypes. In addition, chromosomal banding patterns, karyotype comparisons among wild cowpea species, chromosomal distribution of ribosomal DNA (rDNA), a centromeric repetitive DNA family and Ty1-copia-like retrotransposable elements have been previously reported (Barone and Saccardo 1990; Pignone et al. 1990; Saccardo et al. 1992). While conducting a karyotypic analysis of mitotic chromosomes of 11 wild taxa of *Vigna unguiculata*, Venora and Padulosi (1997) reported a low degree of karyological variability despite the high morphological variability in cowpea.

As in some legumes, such as common bean, 18S–5.8S–25S and 5S rDNA distributions were observed in cowpea (Galasso et al. 1995) which have 18S–5.8S–25S rDNA at chromosomal termini and 5S rDNA proximally located on the same chromosome and have also centromere-specific satellite repeats. The identified Ty1-copia-type retrotransposons are dispersed relatively uniformly across all chromosomes except in centromeres and subtelomeres (Galasso et al. 1997). From molecular cytogenetic studies, Iwata-Otsubo et al. (2016) found very distinct chromosomal structures in cowpea that require further examination. Understanding chromosome structure and the distribution of a few of the major repeat families will help in the ongoing genome sequencing project. In addition, cowpea can benefit from progress made in the cytogenetics of common bean (*Phaseolus vulgaris*) a legume that has the same number of chromosomes and genome size as cowpea (Vasconcelos et al. 2014).

6.4 Molecular Breeding

The advent of genomic revolution, fast advances in genotyping capabilities and bioinformatics provide modern approaches which have the potential to accelerate the development of improved cowpea cultivars. Using conventional approaches requires more than a decade to complete the delivery of improved lines mostly due to several phenotypic evaluations and performance trials characterized by complex, specialized conditions, techniques and skills needed for selection of best lines. Molecular marker-assisted selection (MAS) uses markers that are linked to traits or to estimates of genotypic effects (QTL) instead of phenotypic measurement alone, and it provides a powerful and potentially cost- and time-saving avenues to increase the rates of genetic gain in plant breeding programs (Ehlers et al. 2012). Substantial development of genomic resources in cowpea has occurred recently and some applications of molecular breeding are being recorded in cowpea breeding programs (Fig. 6.3).

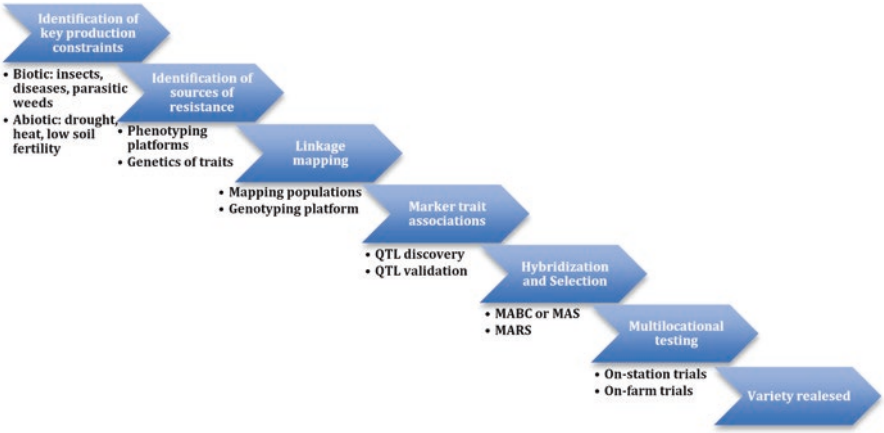

Fig. 6.3 Scheme of molecular breeding in cowpea. (Source: Boukar et al. 2016)

6.4.1 High-Throughput Genotyping Platform

Using methylation filtration (MF) technology, Timko et al. (2008) reported the sequencing and analysis of the gene-rich, hypomethylated portion of the cowpea genome characterized by more than 250,000 gene-space sequence reads (GSRs). The data generated provided an excellent starting point for both marker development and comparative genomics.

Efforts to develop microsatellite (SSR) markers needed by breeders in the implementation of modern breeding have been reported by several research groups. Gupta and Gopalakrishna (2010) have described the identification and development of unigene-based SSR markers in cowpea. These authors characterized and validated 102 SSRs out of 1071 SSRs found in 15,740 cowpea unigene sequences available from the National Center for Biotechnology Information (NCBI) database. Using de novo transcriptomic analysis of cowpea, Chen et al. (2017) identified valuable sets of SSR markers to be validated and used in different genetic and breeding studies.

Through the Generation Challenge Programme's (GCP) Tropical Legumes I project, the University of California Riverside (UCR) and partners developed a high-throughput genotyping platform based on the Illumina GoldenGate Assay for 1536 SNP loci. This resource represents 1536 expressed sequence tag (EST)-derived SNPs (Muchero et al. 2009a). KBiosciences in the UK converted about 1000 mapped SNPs from this platform for use with the single-plex KBiosciences KASPar genotyping platform. This made the platform more readily available, flexible and affordable to the cowpea breeding community (Ehlers et al. 2012).

Recently Muñoz-Amatriaín et al. (2016) reported resources developed from an IITA developed line, IT97K-499-35, and 36 diverse accessions leading to the development of an Illumina Cowpea iSelect Consortium Array, a genotyping assay for 51,128 SNPs.

6.4.2 High-Density Genetic Maps

For the implementation of modern molecular breeding program, consensus genetic linkage maps are key required genomic resources. Boukar et al. (2016) listed several linkage maps developed for cowpea. The first comprehensive cowpea genetic linkage map in terms of number and type of markers was developed by Ouédraogo et al. (2002). The total number of markers on this map is 441. Muchero et al. (2009a) reported the first cowpea consensus genetic linkage map, which consisted of 928 markers spanning 11 linkage groups over a total map size of 680 cM. Genetic maps from 6 RIL populations were merged to build this consensus map. Lucas et al. (2011) reported its improved version with 33% more bins, 19% more markers and had an improved order compared to the first consensus genetic map. Recently Muñoz-Amatriaín et al. (2016) used 5 biparental RIL populations to develop a consensus genetic map having 37,372 SNP loci mapped to 3280 bins with higher average density of 1 bin per 0.26 CM.

6.4.3 Phenotyping and Marker-Trait Association

The implementation of modern breeding requires high-throughput phenotyping platforms. Accurate phenotypic and genotypic data are also needed for the execution of an effective and efficient modern breeding. Screening protocols for both biotic and abiotic stresses require high levels of refinement to facilitate precise data measurements. Cowpea breeding programs are currently using the Breeding Management System (BMS) of the Integrated Breeding Platform (IBP) to design electronic field books that are uploaded into handheld devices (tablets, phones) to be used for data capture. In addition to the tablets, barcoding devices are being introduced in these breeding programs. These different tools will help in the reduction of errors and facilitate timely generation of accurate data. With the advent of advances in molecular marker technologies, precise phenotypic data are being used in combination with genotypic data to identify markers linked to target traits.

Recently, Boukar et al. (2016) reviewed extensively marker-trait association studies reported in the literature for cowpea. Several quantitative trait loci (QTL) were identified using different types of markers (Table 6.2).

6.4.4 Molecular Breeding Deployment

The genomic resources generated during implementation of the Tropical Legumes I project, have led to the initiation of molecular breeding of cowpea. Some of the strategies being adopted include marker-assisted backcross (MABC), marker-assisted selection (MAS) and marker-assisted recurrent selection (MARS).

Table 6.2 Mapping of some cowpea traits

Traits	Marker types	No. markers / QTL	Locations	References
Cowpea golden mosaic virus resistance	AFLP	3	Same linkage group	Rodrigues et al. (2012)
Striga resistance	AFLP	3–6	LG1	Ouédraogo et al. (2001)
	SCAR	2	LG 1	Ouédraogo et al. (2012)
	AFLP/ SCAR	4/1	Same linkage map	Boukar et al. (2004)
Bacterial blight resistance	SNP	3	LG3, LG5, LG9	Agbicodo et al. (2010)
Flower bud thrips resistance	AFLP			Omo-Ikerodah et al. (2008)
Seed size	SSR	6	LG1, LG10	Andargie et al. (2011)
Seed weight	RFLP	2	LG 2 LG6	Fatokun et al. (1992)
Seed weight	SSR	6	LG1, LG2, LG3, LG10	Andargie et al. (2011)
Seed size	SNP	10	LG5, LG7, LG2, LG6, LG8, LG10	Lucas et al. (2013b)
Charcoal rot resistance	SNP/ AFLP	9	LG2, LG3, LG5, LG6, LG11	Muchero et al. (2011)
Heat tolerance	SNP	5	LG2, LG7, LG6, LG10, LG3	Lucas et al. (2013a)
Drought-induced senescence	AFLP	10	LG1, LG2, LG3, LG5, LG6, LG7, LG9, LG10	Muchero et al. (2009b)
Maturity	AFLP	2	LG7, LG8	Muchero et al. (2009b)

Source: Adapted from Boukar et al. (2016)

MABC was conducted at IITA, Nigeria, at the Institut de l'Environment et de Recherches Agricoles (INERA), Burkina Faso, the Institut Sénégalais de Recherches Agricoles (ISRA) Senegal and the Eduardo Mondlane University (EMU) Mozambique, for the introgression of *Striga* resistance (IITA, INERA, ISRA), seed size (INERA, EMU), drought tolerance and nematode resistance (EMU) into local cultivars or improved lines. Given that MABC is now routinely used in modern breeding programs, there is a high expectation that cowpea breeding programs will continue using similar strategies in their improvement activities.

Breeders at INERA and ISRA used MAS to develop improved lines with combined desirable traits from two crosses involving three elite parents, i.e. IT93K-503-1 x IT84S-2246 and Mouride x IT84S-2246, respectively. Using QTL information, an ideotype was constructed by aligning all favorable polymorphic marker alleles from among the two parents (Ehlers et al. 2012). The $F_{2:4}$ breeding lines were genotyped and lines having the maximum number of favorable marker

alleles were selected. Further inbreeding of these lines and selection based on marker content led to phenotypic evaluations for target trait expression and replicated yield tests. Compilation of data needed for preparing the dossier to accompany application for their potential release as new cultivars is being carried out.

Under the TL I project, MARS scheme was also implemented in the participating countries which included Burkina Faso, Senegal, Mozambique and Nigeria. Elite by elite crosses were performed and selection indices based on grain yield and identified associated QTL were used to identify high yielding individuals with complementary favorable marker configurations. Intercross of lines with these complementary markers were performed to pyramid favorable alleles in individual background. After two to three cycles, lines with all favorable alleles were identified and are being tested for possible release.

6.5 Genetic Engineering

6.5.1 Cell and Tissue Culture Approaches

In in vitro plant regeneration two methods commonly used are somatic embryogenesis and organogenesis (Machuka et al. 2002). Plant hormones and other supplements added to the culture medium affect both methods. Several plant tissue culture techniques have been reported in cowpea to attempt to regenerate whole plants from various genotypes (Brar et al. 1999). These efforts are needed to implement gene transfer methodologies. Except for soybean, plant regeneration protocols in grain legumes have not been as reliable as for other crops. This is the reason why grain legumes are described as *recalcitrant crops* to in vitro manipulations (Monti et al. 1997). Concerted efforts were performed to discriminate new buds or induce their multiple proliferations by using different explant sources and several combinations of natural or synthetic plant growth factors.

Shoot Differentiation Using a modified B_5 medium containing coconut water from fresh local coconuts and a high cytokinin concentration, scientists at IITA reported that about 33% of explants (primary leaves and hypocotyl isolated from germinating seeds) differentiated some shoots (Monti et al. 1997). According to these authors, the histology of these explants showed that a strong proliferation occurred on the explant surface, at the epidermal level, where callus was formed. The fact that only the basal parts of young leaflets were able to produce shoots was due to the presence of formed meristems.

Somatic embryogenesis is used for most genetic transformation protocols with recalcitrant legumes given that embryogenic tissues are very prolific and usually originate from single cells (Hansen and Wright 1999). Ganapathi and Anand (1998) reported that induction of somatic embryos occurred in suspension cultures of calli derived from cowpea seedling leaf explants.

Kononowicz et al. (1997) developed a morphogenic system for cowpea using embryonic axis and cotyledonary base explants. Shoot meristem regeneration and morphogenesis were carried out on MS medium (Murashige and Skoog 1962) containing N^6-benzyl adenine (BA) and NAA (naphthalene acetic acid) at different concentrations (10 μM and 5 μM for BA; 0.2 μM and 0.05 μM for NAA) with a pH of 5.8. In addition, media were supplemented with modified B5 vitamins (Gamborg et al. 1968). Through morphogenesis, up to 15 shoots can be regenerated from a single primary explant. Shoots obtained from cotyledon segments and embryonic axes cultures can be easily elongated and rooted. Rooting of cowpea plantlets is most successful in hormone-free MS medium with the addition of 1 mg/L of indole-3-acetic acid (IAA) or 0.05 mg/L of NAA.

Multiple Bud Regeneration Experiments were conducted in order to induce multiple bud proliferation from highly morphogenic cowpea tissues to provide a different approach to plant differentiation with the aim to obtain plants from transformed tissues (Monti et al. 1997). These authors also reported that cotyledon segments and embryonic axes from embryos of different ages of various cowpea genotypes were placed in media containing high concentrations of BAP (3–6 mg/L) and a low concentration of auxin by scientists at Purdue University. Cotyledon explants developed shoots at 50% frequency after 3 weeks of in vitro culture (Monti et al. 1997). They further reported that the herbicide thidiazuron was used as a plant growth regulator to induce multiple bud proliferation from cotyledonary and apical nodes. The best results in terms of frequency of multiple bud proliferation from apices were obtained with cvs. Cornetto and TVu9062 with an average of 87– 85%, respectively.

Multiple shoot formation was attempted through organogenesis from different explants including roots, stem pieces, intact immature cotyledons or protoplasts derived from immature cotyledons, leaves and stem apices (Machuka et al. 2002). At IITA, organogenesis was obtained in several genotypes including IT90K-277-2, IT89KD-288, IT83F442, IT86D-1010, IT93K-624, Vita3 and Ife Brown, when cultured in vitro (Machuka et al. 2002).

These attempts to regenerate cowpea were not successful enough to be used in the genetic transformation of the crop. The first successful genetic transformation of cowpea was reported by Popelka et al. (2006) using regeneration by organogenesis of a wide range of explants on culture media with moderate levels of cytokinin BAP. Longitudinally bisecting seeds through their embryonic axes and by removing both shoot and root apices produced the best explants for multiple shoot formation. These authors have conducted intensive cultivar and culture media comparisons to confirm that (1) there are special media preferences for particular cultivars, (2) the number of independent shoots was not affected by the polyamine growth regulator putrescine (but their development), (3) only deformed shoots could be obtained even at concentrations of thidiazuron as low as 0.1 uM and (4) the effective way to overcome the difficulties in rooting of cowpea was to graft rootless shoots onto cowpea seedlings.

6.5.2 Transformation Systems

In crop improvement, genetic engineering becomes an attractive option when facing intractable problems which conventional breeding methods have not been able to resolve. The legume pod borer (*Maruca vitrata*) causes significant grain yield loss in cowpea if the crop is not protected with an appropriate synthetic insecticide. At IITA and partners breeding programs, concerted efforts were made to identify which among the more than 15,000 cultivated and wild relatives' germplasm lines show resistance to this troublesome insect pest of cowpea. According to Jackai and Daoust (1986), despite availability of a large number of accessions in the cowpea germplasm and cultivars only moderate levels of resistance to the pod borer and pod bugs have been detected. Wild cowpea relatives that show high levels of resistance to the insect pests (Singh et al. 1990) are not cross compatible with the cultivated cowpea (Fatokun 2002). Some *Baccillus thuringinesis (Bt)* protoxins were tested in artificial diets for their efficacies on maruca, the main culprit in damage to cowpea, and *Cry1ab, Cry1C* and *Cry11A* were found to be most potent in curtailing the growth and development of the insect larvae (Machuka 2002). Successful in vitro culture is essential for genetic transformation in plants. Some efforts have been made towards developing a robust genetic transformation system for cowpea with little success. Cardi et al. (1990) isolated protoplasts from cowpea leaves and these were cultured in MS medium with 3% sugar and a pH of 5.8. Plating efficiencies of protoplasts varied with the cowpea lines used. Protoplasts proliferated and formed calli. Roots developed from the calli but no shoots were obtained. Kononowicz et al. (1997) reported successful transformation of cowpea using microprojectile bombardment and cocultivation with *Agrobacterium tumefaciens*. They first tested several cowpea plant parts as explants to establish regeneration, including shoot tips and axillary buds excised from 6–7 day old seedlings. The authors settled for embryonic axis and cotyledonary base as explants. Transient expression of a β-glucoronidase reporter gene was established in the transformed tissues. Organogenesis was observed when transformed tissue was placed in culture medium containing BAP. Popelka et al. (2006) were the first to report successful transformation of cowpea with the *Bt* gene that confers resistance against the larvae of the *Maruca vitrata* pod borer. Of the many cowpea lines tested by these authors for their ability to be genetically modified, only IT86D-1010 showed positive response. Genetically modified cowpea plants carrying the *Bt* gene have been evaluated in confined field trials in Nigeria and Burkina Faso (AATF 2016). Results showed good levels of expression of the gene in the cowpea lines as maruca larvae failed to cause damage to transgenic cowpea plants. The *Bt* gene has been transferred using the backcross method of breeding to some already released cowpea cultivars preferred by farmers in Nigeria. The protocol followed in producing transgenic cowpea, as described by Higgins et al. (2012), was able to transform cowpea with a gene from the common bean (*Phaseolus vulgaris*) that codes for α-amylase inhibitor. Three of four selected transgenic plants showed complete protection of the plants against the cowpea storage pest beetle *Callosobruchus maculatus*. With the

successes reported by Popelka et al. (2006) and Higgins et al. (2012) the genetic transformation of cowpea with desirable genes has become a reality.

6.6 Mutation Breeding

6.6.1 Conventional Mutagenesis

Mutation breeding is most often carried out on already existing elite crop cultivars. This derives from the fact that induced mutations usually affect simply inherited traits such as disease resistance, color, earliness to flower, etc. This method of breeding is therefore embarked upon especially when a desired simply inherited trait is lacking in an improved crop cultivar. The expectations are that induced mutation will result in plants expressing the desired traits. The International Atomic Energy Agency (IAEA) in collaboration with the Food and Agriculture Organization (FAO) supports the implementation of mutation breeding in its member states through the development and utilization of technologies that induce mutations of plants. Among these technologies, gamma irradiation and X-rays are the most commonly used. Adaptation to harsh conditions (drought, salinity, low soil fertility), enhancement of crop nutritional value and resistance to diseases and pests are the usual targets of the supported mutation breeding. Table 6.3 lists the released mutant cowpea cultivars created through the efforts of IAEA. Few reports are available in cowpea cultivar development using induced mutation. Physical and chemical mutagens have been applied for inducing mutagenesis in cowpea. Ojomo (1973) reported gamma radiation treatment caused numerous chromosome disorders as well as high genetic variation for number of pods per plant and grain yield in cowpea. Adekola and Oluleye (2007) irradiated an improved cowpea breeding line IT84S-2246 using two levels of gamma radiation – 196 and 245 Gy. Some plants in the M2 generation from seeds treated with 245 Gy dose were observed to have leaflets terminating in tendrils, broad leaflets, pigmented pods and plant parts and carried the pods above the canopy. Dwarf plants were recovered from the seeds treated with 196 Gy. Girija et al. (2013) irradiated cowpea seeds with gamma rays (20, 25, 30 KR) and as well treated with ethyl methyl sulfonate (EMS) (20, 25, 30 mM). In the M6 generation, several unique mutants were detected. The highest frequency of mutation was observed in flower color among progeny of seeds treated with 25 mM EMS. The most distinct mutants identified were associated with flower color, altered seed size, shape and seed coat color. Horn et al. (2017) irradiated four elite cowpea cultivars in Namibia. Four mutants were selected at M6 generation, which, at M7, showed broad adaptation in the country. They also had the highest grain yield that ranged from 1.95–2.83 mt ha^{-1}. Most of the high performing mutants were derived from seeds of one of the four cultivars (cv. Shindimba) that were irradiated. An observed interesting trait among the mutants was straight pod shape which farmers in the country prefer. Horn et al. (2016) irradiated three improved cowpea cultivars,

Table 6.3 List of released mutant cowpea in different countries

Cultivar name	Country	Registration year	Short description
Uneca-Gama	Costa Rica	1986	Developed by irradiation of seeds with gamma rays (100 Gy). Main attribute of mutant cultivar is high yield
V16 (Amba)	India	1981	Developed by treatment with chemical mutagen DMS. Main attributes of mutant cultivar are high yield, resistance to fungal and bacterial diseases
V37 (Shreshtha)	India	1981	Developed by treatment of seeds with chemical mutagen DMS. Main attributes of mutant cultivar are high yield, high vegetative growth, suitable also as fodder
V38 (Swarna)	India	1981	Developed by treatment of seeds with chemical mutagen DMS. Main attributes of mutant cultivar are high yield, early maturity, synchronous flowering, better quality pods and grains, resistance to diseases
V240	India	1984	Developed by treatment with chemical mutagen DMS. Main attributes of mutant cultivar are high yield, resistance to fungal, viral and bacterial diseases
Co 5	India	1984	Developed by irradiation of seeds with gamma rays (300 Gy). Main attributes of mutant cultivar are more nutritive forage cowpea, high yield (16%), comparable for intercropping with fodder cereals
Cowpea-88	India	1990	Developed by irradiation of F1 generation from cross Cowpea-74 X virus resistant strain H-2. Main attributes of mutant cultivar are high grain yield, high green fodder yield, resistance to yellow mosaic virus
ICV 11	Kenya	1985	Developed by irradiation of seeds with gamma rays. Main attributes of mutant cultivar are semi-erect, large leaves, green stem, green pods, maturity in 65 days, yield 1100 kg/ha, resistance to cowpea aphid
ICV 12	Kenya	1985	Developed by irradiation of seeds with gamma rays. Main attributes of mutant cultivar are higher yield than ICV 11 and resistance to cowpea aphids
TRC77-4 (Kalleshwari)	India	2007	Improved traits: yield
COCP 702 [=CoVu 702 & CO(CP) 7]	India	2002	Developed by irradiation of seeds with gamma rays (200 Gy). Main improved attributes of mutant cultivar are high yield and good quality
Gujarat cowpea-1	India	1984	Improved traits: yield, early maturity, root knot resistance

(continued)

Table 6.3 (continued)

Cultivar name	Country	Registration year	Short description
CBC5	Zimbabwe	2017	Developed through mutation induction using gamma irradiation of CBC1 seeds at 150 Gy. Main improved attributes are high grain and fodder yield (at least 18% yield advantage), good dynamic stability across environments (sites and seasons) and increased seed size (at least 8% seed size increment over parent)

Source: Adapted from Joint FAO/IAEA Mutant Varieties Database (2019)

namely IT81D-985, IT89KD-245-1 and IT87D-453-2, and detected substantial genetic differences among these cowpea genotypes following mutagenesis. Differences in flowering ability, days to maturity, flower and seed colors and grain yields were observed among the mutants. They identified and isolated ten phenotypically and agronomically stable novel mutants in M6 generation from each of the three cultivars. These promising mutant lines were recommended for tests across several agro-ecologies for their adaptability. Those with superior performance were to go for large-scale production or used as parents in the Namibian cowpea breeding program. In Nigeria, Odeigah et al. (1998) treated seeds of two cowpea breeding lines with three mutagens: gamma radiation, EMS and NaN_3. The three lines responded differently to the mutagens. As expected, the mutagens had both desirable and deleterious effects on the plants resulting from treated seeds. An array of mutants was observed among plants in the M2. The observed mutants showed differences in plant morphology and physiology. They further reported that induced mutations resulted in plants with branched peduncles, pigmentation of pods and plant parts, male sterility, early maturity, as well as resistance to cowpea storage weevils and aphids. Some other observable traits among the mutant plants were stunting in growth, twining stem and spreading growth habit none of which is present in any of the untreated lines. The protein content in grains of some mutants were found to be higher by up to 13.3%, in a mutant from IT84E-124, and 13.64% from Vita 7, when treated with 1.0 mM NaN_3. Olasupo et al. (2016) applied UV light on cowpea pollen with the aim of inducing mutations. The results showed that for the duration of exposure of pollen to the UV rays no visible morphological change was induced in the progeny derived from seeds using the pollen for pollination.

In India at least seven cowpea cultivars improved using mutation breeding procedures were released between 1981 and 2007 at the Bhabha Atomic Research Centre, Trombay, (Punniyamoorthy et al. 2007). These mutant cultivars are characterized by high grain yield and early or medium maturity. Others have increased seed size, resistance to yellow mosaic virus or increased fodder yield. From the foregoing, it is evident that cowpea cultivar development has benefited from mutation breeding in different countries and the cultivars have in most instances met the expectations of farmers and consumers.

In cowpea, seeds and pollen grains have been treated with chemical and physical mutagens for the purpose of inducing mutations in the crop. There is however no report on the induction of mutagenesis in cowpea using in vitro cultured plant tissues. This is not surprising because totipotency is very difficult to achieve in cowpea. All of the reported transgenic events in cowpea have been carried out by coculturing existing plant tissues with *Agrobacterium* carrying the genes of interest (see Sect. 6.5 on genetic engineering above).

Ojomo (1973) reported that exposing cowpea seeds to gamma radiation resulted in numerous chromosomal disorders. However, most of the other above reports on mutation induction in cowpea did not mention whether the mutation induced intergenic (occurring within the DNA) or structural i.e. intragenic disorders that occur on the chromosome such as deletions, inversions, translocations or duplications, changes in chromosome numbers such as polyploidy, aneuploidy or haploidy. In a review of mutation breeding in different crops, Oladosu et al. (2016) concluded that base substitutions, a term implying nucleotide changes involving substitution of one base for another, are among the different types of mutations at the molecular level. According to these authors, base substitutions can happen through mis-pairing during replication of the base analogue in the treated DNA.

6.7 Hybridization and Heterosis

Cowpea is a highly self-pollinated crop. The cleistogamous flowers, which open for only 1 day, are large and showy which make hybridization relatively easy. The level of outcrossing in cowpea is generally low (Fatokun and Ng 2007) and could vary with the environment where the crop is grown. The anthers open to release pollen grains contained therein on the day the flower opens. The stigma is however receptive from a day before anthesis and remains so until day of flower opening. Insects, especially bumble bees, are the major cowpea pollinator. There is no clear evidence that color influences insect visits to flowers in cowpea. Leleji (1973) reported that bumble bees tended to visit purple colored flowers more whereas honey bees visited white colored flowers more frequently.

Strong cross-incompatibility exists between cowpea and many of its wild relatives, particularly those outside the section Catiang. Even among members of section Catiang difficulties are encountered when making crosses between some of them. For example, Fatokun and Singh (1987) had to use embryo rescue to enhance crossing between cultivated cowpea and *Vigna unguiculata* ssp. *pubescens*. However, the F1 hybrids showed partial fertility. All attempts so far made to cross *V. vexillata* to cowpea have not yielded positive results. *Vigna vexillata* has genes that confer resistance to many of the insect pests that cause serious yield losses in cowpea. Barone and Ng (1990) examined the causes of the incompatibility between cowpea and *V. vexillata*. They observed that pollen tube growth through the styles in interspecific crosses was arrested in stigmatic tissues and only 15–20% of ovules were fertilized. Embryos developed following interspecific hybridization did not go

beyond the globular stage before they started to collapse. Even when pods resulting from interspecific hybridizations were retained on the plants until maturity subsequent treatment with auxin did not result in any success (Fatokun 2002).

Varying levels of heterosis have been reported in cowpea. Agble (1972) reported seed size heterosis in crosses made between four local Ghanaian cowpea cultivars. Bhaskaraiah et al. (1980) in a 10 × 10 set of diallel crosses found relatively high heterosis for grain yield and pods per plant but lowest for 100-seed weight. Bhushana et al. (2000) generated 36 hybrids from line tester crossing design and found mid-parent heterosis of 112.4% for pods/plant, 105.32% for seed yield per plant and 30.31% for pod length. Mak and Yap (1977) evaluated heterosis for protein content in a diallel cross involving seven cultivars of yardlong bean. Heterosis ranged from 40.7% to 63.2% but only three F1 hybrids showed significantly higher parent values. Number of pods per plant showed the highest heterosis among yield components while seeds per pod, seed weight and pod length showed relatively low levels of heterosis. In spite of the reported levels of heterosis in cowpea no record is available of any hybrid cultivar released for cultivation. The drawback to developing hybrid cultivars in cowpea can be attributed to the high level of self-pollination in the crop. In addition, genetic but not cytoplasmic male sterility has been reported in cowpea (Ladeinde et al. 1980; Sen and Bhowal 1962). Cytoplasmic male sterility would be advantageous to a successful hybrid cultivar development program in cowpea.

6.8 Conclusions and Prospects

Cowpea possesses a high potential to play a strategic role in tackling the complex challenges of hunger, malnutrition, environmental sustainability, climate change and increasing food prices, confronting the global community in coming decades (Widders 2012). In the dry Sahel and Savannah areas where the crop is being largely produced, increasing human population is threatened by food and nutritional insecurity. Cowpea can contribute to alleviate hunger and malnutrition as it has demonstrated its ability to grow in these regions and to be the cheapest source of plant protein. Cowpea fodder also plays an important role as animal feed. Haulms constitute nutritious feed for animals, most especially for ruminants. Another characteristic of cowpea is its ability to fix atmospheric nitrogen through symbiosis with bacteria living in its root nodules. This contributes to the sustainability of the agricultural systems. The soils in the cowpea-growing areas are greatly degraded by the inability of farmers to provide adequate amounts of fertilizers to ameliorate the problem of poor soil fertility. Numerous factors including biotic and abiotic factors are limiting the production of this important crop. It is necessary that adequate attention be given to addressing the constraints that have continued to hamper the productivity of cowpea in the fields of SSA farmers, as this will enhance the sustenance of the contributions of the crop to food and nutrition security. Lack of sources of genes for some traits of interests (e.g. insect resistance) is the major limitation to

fully implementing conventional breeding in cowpea. In addition, conventional breeding is ineffective in realizing adequate genetic gain that could allow farmers in SSA to benefit optimally from cowpea production. The advent of new technologies and approaches such as molecular breeding and gene editing offer opportunities to modernize cowpea improvement programs. In recent times, several donor communities have advocated and supported the implementation of modern breeding approaches in the Consultative Group on International Agricultural Research (CGIAR) centers and some National Agricultural Research Services (NARS) programs. Cowpea programs at IITA and NARS are currently involved in the modernization of breeding approaches. With the continued support of these initiatives, there is hope that cowpea breeding programs will soon become more efficient and effective and sustainable production of cowpea will therefore be significantly increased in SSA.

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Appendices

Appendix I: Main Research Institutions Relevant to Cowpea

Institution	Area of specialization	Research activities	Contact information including website
International Institute of Tropical Agriculture (IITA)	Genetic Improvement	Activities were carried out in order to develop improved lines with (1) high yield potential, (2) resistance /tolerance to both biotic and abiotic stresses, (3) good adaptation to monocropping and intercropping systems and (4) grain characteristics preferred by consumers and processors	IITA Kano o.boukar@cgiar.org IITA Ibadan c.fatokun@cgiar.org http://www.iita.org
	Natural resource management	Activities were carried out for development of useful methods to supply P nutrient on cowpea by using local materials, e.g. rock phosphate and organic matters. In addition, the utilization of arbuscular mycorrhizal fungi is also being tested as P uptake enhancer by cowpea	IITA Lusaka Zambia k.suzuki@cgiar.org http://www.iita.org

(continued)

Institution	Area of specialization	Research activities	Contact information including website
	System Research / Agronomy	Development of (1) village-based/commune-based dissemination scheme for improved cowpea (AVEC-BF), (2) single-seed protein content evaluation technique, (3) high protein content management technique, and evaluation of yield-gap analysis in Burkina Faso	IITA Ibadan Nigeria h.ishikawa@cgiar.org http://www.iita.org
	System Research / Agronomy	Conduct research to identify best crop management (Planting dates, population, cropping sequence, fertilizer use) techniques that exploit the potential of improved cowpea cultivars and close the yield gap between what is obtained in the research stations and farm fields. Evaluate performance of cowpea cultivars in both sole and different intercropping systems since most cowpea is grown in intercrops in West and Central Africa. The use of cropping systems models to simulate cowpea growth and yield in diverse ecologies and cropping systems and management practices is evaluated in the Agronomy unit	IITA Kano a.kamara@cgiar.org http://www.iita.org
	IPM research	Pest management research focusing on West Africa is addressing critical insect pests such as the legume pod borer *Maruca vitrata*, for which a range of bio-pesticides and biological control agents have shown promising results. For aphids and thrips, novel sources of host plant resistance from the IITA-mini core are currently being evaluated, and can be integrated with biological control approaches	IITA Cotonou Benin m.tamo@cgiar.org IITA Kano a.togola@cgiar.org http://www.iita.org

(continued)

Institution	Area of specialization	Research activities	Contact information including website
	Genetic Resource	The IITA gene bank holds the world's largest and most diverse collection of cowpeas with 15,122 unique samples from 88 countries, representing 70% of African cultivars and nearly half of the global diversity and about 2000 accessions of cowpea wild relatives. Activities undertaken with IITA breeders include genotyping of the core collection (with University of California Riverside) and development of a trait based subset for drought and heat tolerance (with ICARDA). Wild relatives are being evaluated under a project supported by the Global Crop Diversity Trust	IITA Ibadan m.abberton@cgiar.org
	Socio-economics	Comprehensive household and plot level surveys are conducted in order to assess the adoption and ex-post impacts of improved cowpea cultivars on yield, farm income, food security and poverty. Gender differentials in adoption and impacts are also taken into consideration when conducting these assessments. Quantify ex ante poverty impact of improved cowpea technologies across drylands of Sub-Saharan Africa and South Asia	IITA Malawi a.alene@cgiar.org j.manda@cgiar.org s. gbegbelegbe@cgiar. org
Institut de l'Environnement et des Recherches Agricoles (INERA), Kamboinse and Saria, Burkina Faso	Genetic Improvement	Activities were carried out in order to develop improved lines with (1) high yield potential, (2) resistance /tolerance to both biotic and abiotic stresses, (3) good adaptation to monocropping and intercropping systems and (4) grain characteristics preferred by consumers and processors using classic and modern breeding tools	INERA/ CREAF-Kamboinse batieno52@gmail.com INERA/DRREA-Sariahamasegua22@ gmail.com

(continued)

Institution	Area of specialization	Research activities	Contact information including website
Institut de Recherche Agricole pour le developpement (IRAD), Maroua, Cameroon	Genetic Improvement	Activities were carried out in order to develop improved lines (1) well adapted to the Soudano Sahelian zone of Cameroon, (2) high yield potential and preferred by farmers, (3) resistance / tolerance to pest weeds (*Striga*), insects (aphids, thrips) and diseases (*Colletotrichum*)	IRAD Maroua (Cameroon) sobdagonne@gmail.com http://www.iradcameroun.org
Savanna Agricultural Research Institute (SARI), Tamale, Ghana	Genetic Improvement	Activities includes, developing improved lines with (1) high yield potential, (2) resistance /tolerance to both biotic and abiotic stresses, (3) good adaptation to monocropping and intercropping systems and (4) grain characteristics preferred by consumers and processors using both molecular and conventional techniques. Germplasm collection and evaluation. Yield evaluation of advance elite lines across multilocations and cultivar release and maintenance	Haruna_ mohammed67@yahoo.com Owusu owusuemmagh@yahoo.com Francis Kusi onkufra@yahoo.com
	On-farm Agronomy	Activities were carried out to test the performance of elite promising lines for release under the farmers' own environment and management	Julius Yirzagla yirzagla@yahoo.com Mahama Goarge Yakubu mgyakubu@yahoo.com
	IPM	Pest management research activities in Ghana include evaluating cowpea engineered with cry genes from *Bacillus thuringiences* for control of the legume pod borer *Maruca vitrata*, survey of alternate host plants for *M. vitrata* as possible refugia for IRM in Bt-cowpea, evaluating cultivars for host plant resistance to thrips *Megalurothrips sjostedti* and also evaluating biorational botanical pesticides as a cheap source of pesticides for control of major insect pests of cowpea	Mumuni Abudulai mabudulai@yahoo.com S. K Asante skasante@yahoo.com

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Institution	Area of specialization	Research activities	Contact information including website
Institut d'Economie Rurale (IER), Cinzanna, Mali	Genetic Improvement	Breeding activities carried out in order to develop improved lines with:(1) high yield potential, (2) resistance /tolerance to both biotic and abiotic stresses, good adaptation to monocropping and intercropping systems and – 4 grain characteristics preferred by consumers and processors	SRA Cinzana diallo.sory@yahoo.fr
	Genetic Resource	The IER gene bank is not fully functioning. About 2000 accessions for the nine mandate crops (cowpea, sorghum, millet, groundnut). The cowpea mini gene bank is holding the largest and most diverse collection with more than 1000 accessions from different agro-ecologies in Mali	SRA Cinzana mousmandiaye@yahoo.fr
	IPM research	Identification of source of insects and disease resistance (parental lines) for cultivar development	SRA Cinzana zkouyate@yahoo.fr
	Agronomy	Conduct research to identify crop management (planting dates, population, fertilizer use). Evaluate performance of cowpea cultivars in sole and different intercropping systems since most cowpea is grown in intercrops in Mali	SRA Cinzana kmarcel59@yahoo.fr
	Seed production Unit	Production of breeder and foundation seeds to supply private seed companies; production of certified seeds to supply farmers	SRA Cinzana mousmandiaye@yahoo.fr
Institut National de la Recherche Agronomique du Niger (INRAN), Niamey, Niger	Genetic improvement	Creation of high yielding cultivars (grains, fodder) with tolerance to major biotic and abiotic stresses, adapted to mixed cropping	souleymanabdou@gmail.com masalif2000@yahoo.fr
	Socio-economics	Adoption studies, impact assessment	geribro@yahoo.fr
		Value chain	bokarmoussa@gmail.com
	Seed production	Production of foundation seeds to supply private seed companies; Production of certified seeds to supply farmers	salamiissoufou@yahoo.fr

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Institution	Area of specialization	Research activities	Contact information including website
	IPM	Laboratory and field screening of cultivars for tolerance to insects; Chemical and biological control of insects; Improved storage technologies	amadoulaouali@gmail.com
	Genetic resources	Management of the gene bank	issazakarym@yahoo.fr
Institute for Agricultural Research (IAR), Nigeria	Genetic Improvement	Activities were carried out in order to develop, release and register cowpea cultivars with (1) high yield potential,(2) resistance /tolerance to both biotic and abiotic stresses, (3) good adaptation to mono and intercropping systems	IAR Samaru Zaria mahammadlawan@yahoo.com binsaba@yahoo.co.uk mffaguji@hotmail.com
	Agronomy	Develop production guides in line with best recommended agronomic practices of the newly developed, and commercially released cowpea cultivars for recommendation to farmers	sojiolufajo@yahoo.com https://iar.abu.edu.ng/
	IPM research	Help in identifying source of insects and disease resistance (Parental lines) for cultivar development. Also, develop an Insects Resistance Management (IRM) plan(s) for an insect resistance cowpea cultivars developed for commercial use in Nigeria.	imutono@yahoo.com rsadamu@yahoo.com
	Genetic Resource	The IAR gene bank (currently not fully functioning) is designed to holds about 35,000 accessions for the nine mandate crops (Cowpea, Groundnut, Maize, Sorghum, Cotton, Castor, Jatropha, Sunflower and Artemisia). The cowpea mini gene bank is holding the country's largest and most diverse collection of cowpeas with more than 5000 accessions from 6 Nigerian agro-ecologies and 10 African countries, representing 60% of Nigerian cultivars, 40% of African cultivars and nearly 10% of the global diversity.	mahammadlawan@yahoo.com uwa6474@yahoo.com

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Institution	Area of specialization	Research activities	Contact information including website
	Seed production Unit	The IAR seed Production Unit is mandated for the production of foundation and certified seeds of the seven crops: cowpea, groundnut, maize, sorghum, cotton, castor, sunflower	almuh2013@yahoo.com
	Product Development Unit	The program is mandated for conducting researches: to determine the nutritional and biological value of released and candidate cultivars; assess the suitability of the cultivars for industrial processing and to improve the technology of local food processing. Also, monitor foods and feeds for toxic contaminants. The program participate generating new technologies and in training of both individuals and organization that are interested in using the technologies generated by the program	sanbugaje@gmail.com https//iar.abu.edu.ng/pages/prodctdeveloprech.html
	Socio-economics	Participatory Rural Appraisal for trait prioritization and inclusion in breeding objective, Adoption studies and Impact assessment	m.bellohassan@gmail.com
Federal University of Agriculture, Makurdi (FUAM), Nigeria	Genetic Improvement	Breeding activities carried out in order to develop improved lines with: (1) high yield potential, (2) resistance /tolerance to both biotic and abiotic stresses, (3) good adaptation to different cropping systems and (4) grain characteristics preferred by consumers and processors	FUAM uam.edu.ng lomoigui@cgiar.org lateeflekan@gmail.com
Institut Senegalais de Recherches Agricoles (ISRA), Bambey, Senegal	Genetic Improvement	Develop high yielding cowpea cultivars adapted to semiarid zones with resistance to relevant biotic constraints and good grain quality.	ndiaga.cisse@isra.sn ncisse@refer.sn www.isra.sn
	Pathology	Disease management, host plant resistance	sarrapenda@hotmail.com
	Entomology	Pest management, host-plant resistance	sardr@yahoo.com
	Genetics	Management of Genetic resources	miamybo@yahoo.fr
	Agronomy	Production management	aliouselbe11@yahoo.fr
	Seed production	Manage foundation seed unit	taffaguey@yahoo.fr

Appendix II: List of Main IITA Released Cultivars in Different Countries

Cultivars	List of countries	Main characteristics
TVx 3236	Togo, Uganda, Yemen, Angola, Botswana, Burkina Faso, Cameroon, Liberia, Mali, Mauritius, Nigeria, Senegal, Sierra Leone,	Scab resistance, *Cercospora*, brown blotch, thrips tolerance
IT82D-889	Philippines, Suriname, Somalia, Sri Lanka, Swaziland, Tanzania, Thailand, Zambia, Belize, Bolivia, Guinea Bissau, Liberia, Malawi	Extra early maturity, combined resistance to cowpea yellow mosaic and black eyed cowpea mosaic
IT82E-16	Egypt, Ethiopia, Ghana, Guinea Conakry, Lesotho, Malawi, Mozambique, South Africa, Eswatini (Swaziland), Zambia	Early maturity, Combined resistance to *Cercospora* leaf spot, brown blotch and anthracnose
VITA-3	Belize, Brazil, Fiji, Jamaica, Sierra Leone, Thailand, Venezuela,	Combined resistance to *Cercospora* leaf spot, brown blotch, anthracnose, bacterial pustule, bacterial blight, cowpea yellow mosaic virus and cowpea and black eyed cowpea mosaic
VITA-4	Central African Republic, Haiti, India, Liberia, Myanmar, Sir Lanka,	Resistant to bacterial blight, brown blotch, *Septoria*, scab and root knot
IT84S-2246-4	Benin, Guinea Conakry, Jamaica, Nigeria, USA	Early maturity, bruchid tolerant, scab resistance, root knot nematode resistance, aphid resistance
IT99K-573-1-1	Ghana, Niger, Nigeria, Sierra Leone, Tanzania	Medium maturity, resistance to *Striga* and *Alectra*, stem rot resistance
IT82E-32	Ethiopia, Lesotho, Sierra Leone, Somalia	Early maturity
IT99K-573-2-1	Ghana, Nigeria, Burkina Faso, Sierra Leone	*Striga* resistance, *Alectra* resistance
VITA-5	Central African Republic, Liberia, Togo, Yemen	Field tolerant to leafhoppers, resistant to anthracnose, bacterial pustule and *Cercospora*
IT82E-18	Australia, Belize, Central African Republic, Eswatini (Swaziland)	Early maturity
IT98K-205-8	Burkina Faso, India, Nepal, Niger	Early maturity, *Striga* resistance,
IT81D-994	Cameroon, Central African Republic, Nigeria	Medium maturity, bruchid tolerance, *Striga* resistance for race 4 and race 1, combined resistance to *Cercospor*a leaf spot, brown blotch and anthracnose, *Septoria* leaf spot resistance, drought tolerance
IT82D-789	Sri Lanka, Suriname, Yemen	early maturity,

(continued)

Cultivars	List of countries	Main characteristics
IT83S-818	Central African Republic, Ghana, Mali	Medium maturity, combined resistance to cowpea yellow mosaic, blackeye cowpea mosaic and many strains of cowpea aphid borne mosaic
IT86D-1010	Paraguay, Sierra Leone, Sri Lanka	Early maturity, combined resistance to cowpea yellow mosaic, black eyed cowpea mosaic and cowpea aphid borne mosaic, black eyed cowpea mosaic and 5 strains of cowpea aphid borne mosaic.
IT87D-885	Equatorial Guinea, Haiti, Lesotho	Combined resistance to *Cercospora* leaf spot, brown blotch, anthracnose, bacterial pustule, bacterial blight
IT89KD-374	Mali, Niger, Nigeria	Early maturity, combined resistance to *Cercospora* leaf spot, brown blotch, anthracnose, bacterial pustule, bacterial blight, adapted to intercropping
IT90K-277-2	Cameroon, Nigeria, South Sudan	Combined resistance to cowpea yellow mosaic, cowpea aphid borne mosaic, black eyed cowpea mosaic, cowpea mottle, cowpea cucumber mosaic, combined resistance to *Cercospora*, brown blotch, adapted to intercropping
IT97K-499-35	Mali, Niger, Nigeria	Early maturity, *Striga* resistance, *Alectra* resistance, resistance to scab, brown blotch, *Cercospora*

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Chapter 7
Faba Bean (*Vicia faba* L.) Breeding

Xuxiao Zong, Tao Yang, and Rong Liu

Abstract Faba bean (*Vicia faba* L.) is an important cool season legume grown widely in the world due to its palatability as well as its ecological and environmental value in sustainable agriculture and cropping system. As its protein content is higher than other common food legumes, it is mainly harvested in the form of dry seeds for human food and for animal feed worldwide, but its fresh seeds or pods are often used as vegetables in China, India and other countries with rapidly expanding areas. The dry grain, fresh seeds and sprouts of faba bean are a highly nutritional food source for the human diet. Fresh faba bean seeds are used for a variety of savory dishes, and dry grain are used for paste and snacks, while sprouts for traditional food. The dried fresh stems and leaves of faba beans are good fodder for cattle, sheep and pigs. Faba bean flowers contain a large amount of L-DOPA and can be used to make flower tea. Various faba bean cultivation practices like intercropping and rotation are described. Germplasm diversity and conservation studies on faba bean genetic resources, in vitro regeneration and genetic transformation studies of faba bean, are summarized. Simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) have been developed for faba bean genetic linkage map construction and quantitative trait loci (QTLs) analysis of important genes, are discussed. Achievements of breeding and the future breeding objectives like winter hardy, heat tolerance, herbicide resistance, double-zero, machine sowing and harvesting, biological nitrogen fixation efficiency, photosynthetic efficiency, flavor and palatability, dual usage and market price are reviewed. Current research initiatives and recommendations for future research, like gene editing, are also illustrated.

Keywords Breeding · Dry grain · Faba bean · Quality · Resistance · Vegetable

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7.1 Introduction

Faba bean (*Vicia faba* L.) is a grain legume crop of the Fabaceae family with a long history of cultivation in temperate regions of the northern hemisphere (Duc et al. 2015). Faba bean is a rich source of protein, fiber, and other non-nutrient bioactive compounds (L-DOPA, favin, saponins, tannins) considered beneficial for health (Multari et al. 2015). Its protein content is higher than other common food legumes (Burstin et al. 2011; Griffiths and Lawes 1978). Sometimes called fava, broad bean or horse bean, faba beans are mainly harvested as dry seeds for human food or animal feed globally, but their fresh seeds or fresh pods are often used as vegetables in China, India and other countries. Faba bean is one of the most widely grown cool season legume after pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medk.) globally (FAOSTAT 2018). Faba beans provide valuable ecological and environmental services for sustainable agriculture, a variety of delicious and nutritional food, a diversity of planting systems and many related organisms, including pollinators (Duc et al. 2015; Zong et al. 2019). Faba bean has advantages over other legumes such as soybean in cool season environments being adapted to grow under low temperatures (Fouad et al. 2018). As such, it is well suited to sustainable farming practices in temperate to cool environments (Temesgen et al. 2015). Like other legumes, faba beans play a critical role in cereal-based farming systems, for improving soil fertility (Jensen et al. 2010). The symbiosis of the species with specific rhizobium bacteria leads to biological nitrogen fixation, which effectively reduces fertilizer input to the arable land (Duc et al. 2015). Faba bean has an intrinsic ability to adapt to diverse climates; however, its yield is unstable due to biotic and abiotic stresses, as is the case with many other major legumes (Cernay et al. 2015). In addition, the total grain yield of faba bean was positively correlated with the protein content of the dry grain (El-Sherbeeny and Robertson 1992).

The cultivation of faba bean can be traced back to the origin of agriculture (Cubero 1973), and it is still an important crop today due to its high-yield potential, nutrient-intensive grain (Fouad et al. 2018) as dry seeds and a fresh vegetable, as well as its role as forage and cover crop. The global faba bean harvested area was 2.4 million ha in 2016, and the total production in 2016 was 4.46 million mt of dry grain (FAOSTAT 2018), covering wide range of latitude from about 40°S to 50°N and from sea level to an altitude of 3000 m (Gnanasambandam et al. 2012). Over the past few decades, the global productivity of dry faba bean remains relatively stable (Fig. 7.1), as well as that in China (Fig. 7.2). However, the global area of dry faba bean cultivation has been declining, especially in China (Fig. 7.2) and countries in North Africa and West Asia (FAOSTAT 2018; Fouad et al. 2018). This reflects a general trend observed since the 1960s that farmers are increasingly relying on nitrogen fertilizer as a source of nitrogen inputs (Crews and Peoples 2004), and due to the achievements in faba bean breeding globally.

The major producing countries of dry faba bean in harvesting area are China, Ethiopia, Australia, Morocco, France, UK, Sudan, Tunisia, Peru and Italy; in year 2016, covered 33.57, 17.79, 14.58, 3.44, 3.24, 3.13, 2.95, 2.39, 2.24 and 2.09% of

Fig. 7.1 The harvesting area and productivity of dry faba bean production in the world. (Source: FAOSTAT 2018)

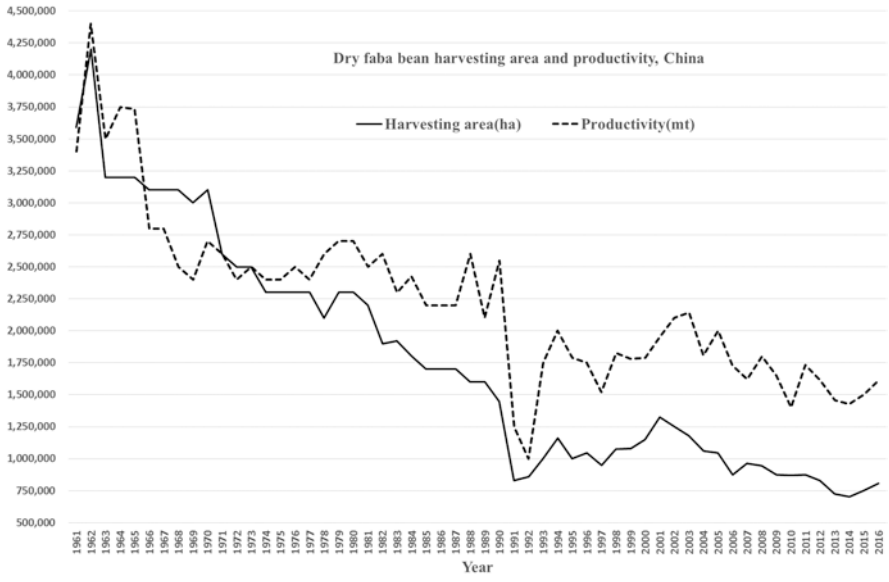

Fig. 7.2 The harvesting area and productivity of dry faba bean production in China. (Source: FAOSTAT 2018)

world share, respectively, covered 85.43% of the global production in total (Fig. 7.3). While, the major countries for dry faba bean productivity in the world are China, Ethiopia, Australia, United Kingdom, France, Germany, Sudan, Egypt, Italy and Peru; in year 2016, covered 36.08, 19.69, 9.50, 6.48, 4.45, 3.45, 2.72, 2.67, 2.24 and 1.61% of total global productivity, respectively, with a total coverage of 88.88% (Fig. 7.4) (FAOSTAT 2018).

In China, vegetable faba bean was cropped on 0.4277 million ha (6.416 million mu) in 2014 (Li et al. 2017); there are no accurate statistics on the production of fresh seeds as vegetables because other producers of faba beans are mainly small scale producers (Duc et al. 2015), except in China.

The seed production system of faba bean is weak; biological stress (leaf diseases and broomrape *Orobanche* spp.) and abiotic stress (heat, cold, drought, acidic soil, waterlogging) limit the development of faba bean production; moreover, lack of effective herbicides and poor adaptability to agricultural mechanization make weed control very difficult (Fouad et al. 2018). However, despite the problems affecting the production of faba beans, the average global yield has increased from 0.9 mt/ha in 1961–1964 to 1.86 mt/ha in 2016 (FAOSTAT 2018). Researchers around the world have made major achievements in genetic improvement of faba beans.

This chapter reviews and highlights the progress and achievements made in understanding the origin of the cultivated faba bean, the achievements in its genomics, breeding and genetic diversity, the advances in molecular genetics and breeding methodologies on breeding for resistance (tolerance) to major biotic and abiotic stresses as well as for yield and market value. Together, these will help to speed up the breeding efforts to improve targeted traits of faba bean.

7.1.1 Origin and Distribution

Faba bean (*Vicia faba* L.) (2n = 2x = 12), with a genome size of approximately 13,000 Mb (Johnston et al. 1999), is a close relative of *Narbonensis* (2n = 14) within subgenus *Vicia*, although they have different chromosome numbers and nuclear DNA content (Kew 2017). Faba bean became a model species for plant cytogenetics in the 1970s and 1980s because it has a small number of chromosomes (6) and are so large that they are easy to observe (O'Sullivan and Angra 2016). Useful agronomical characters such as winter hardiness, resistance to black bean aphid (*Aphis fabae* Scop.) and chocolate spot disease caused by the fungus *Botrytis fabae* Sard. are found among *Narbonensis* species (Birch et al. 1985). Unlike the case in other legume crops, there is no successful record of interspecific crosses between *V. faba* and other *Vicia* species (Caracuta et al. 2016). Faba bean has a long history of cultivation and is widely distributed in different environments. Faba bean is often cross-pollinated; crossing and the response to human selection have given it wide-ranging variation in seed shape, size and color; leaf size and shape and plant architecture (Fouad et al. 2013). Seed size of *Vicia faba* has a range of more than one order of

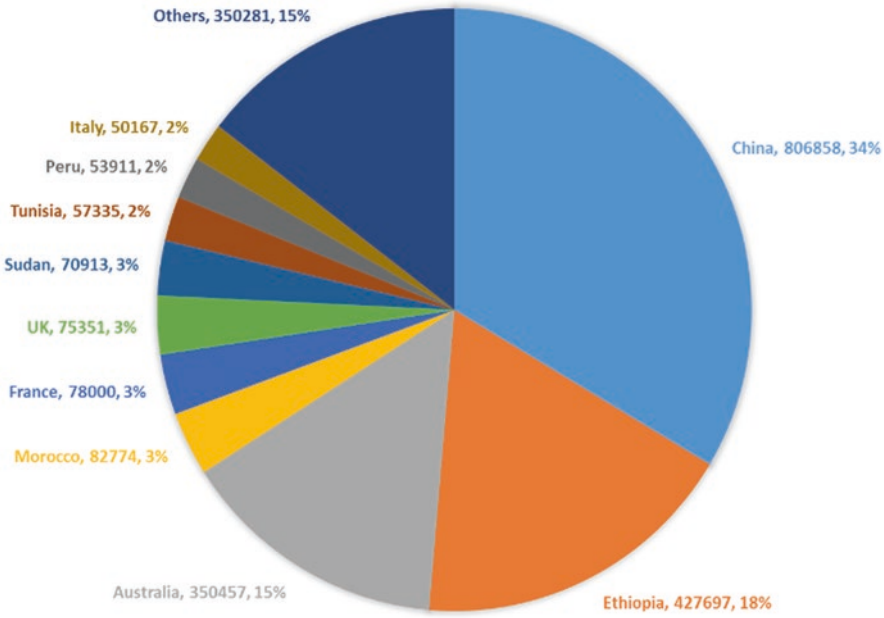

Fig. 7.3 The harvesting area (ha) of top ten producers in the world for dry faba bean production in 2016

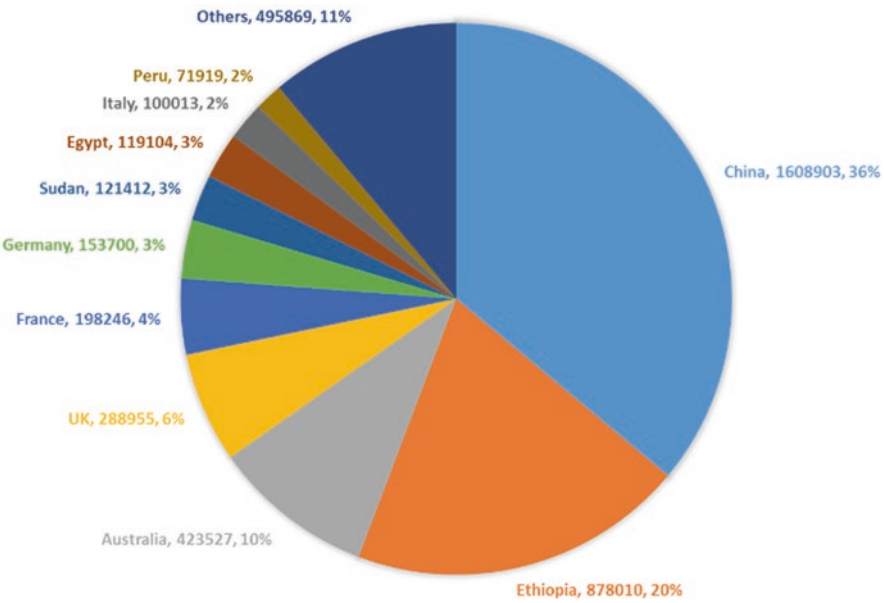

Fig. 7.4 The productivity (mt) of top ten producers in the world for dry faba bean production in 2016

magnitude (20–250 g/100 seeds), making it the largest range in seed size of any angiosperm. Fouad et al. (2018) divided the seeds into three groups: (a) small-seeded type (in southwest Asia), (b) large-seeded type (in the western world) and (c) medium-seeded type (very old and dating back to Neolithic agriculture). Medium-grained species are distributed over a wide area from Spain to the Himalayas (Muratova 1931). Archaeological evidence from Tell Ain el-Kerkh in northwestern Syria indicated that it was the region where faba beans originated and were domesticated in the late tenth century BC (Tanno and Willcox 2006). In addition, 14,000-year-old specimens found in the Mount Carmel region of present-day Israel have been identified as the *lost* ancestors of faba bean (Caracuta et al. 2016).

Muratova (1931) proposed to divide *Vicia faba* into 4 subspecies according to seed size, as *major* (large-seeded type), *equine* (medium-seeded type), *minor* (small-seeded type) and *paucijuga* (small-seeded type). Hanelt (1972) proposed a new classification system (Fig. 7.5), and assigned *paucijuga* as one of the *minor* subspecies of a geographical race and suggested that faba bean had two subspecies *minor* (the oldest) and *faba*. The two varieties of *faba* are *equina* and *faba*. The *minor* variety is divided into *minor* and *tenuis*, *equina* is divided into *equina* and *rugosa*, and *faba* is divided into *faba* and *clausa*.

However, since there is no genetic or sterility barrier between these subspecies, Cubero (1973) believed that they were a single species consisting of four different plant groups. Larger-seeded faba bean are thought to be the result of human selection (Tanno and Willcox 2006). The medium-sized ones were found in the Iberian Peninsula including both Spain and Portugal, and in Central Europe, 5000 years ago. Larger flat-seeded faba beans were not known until 1500 years ago (Ladizinsky 1998).

In the context of cultivar diversification within *Vicia faba*, studies were conducted based on morphological characteristics (Abdalla 1976) and isozyme markers (Jaaska 1997; Polignano et al. 1999) of the variation of selected traits, but revealed no significant discovery of species origin. However, the molecular markers can be used to distinguish the *V. faba* cultivars in different geographical areas. For example, groups of genetic resources can be distinguished by amplified fragment length

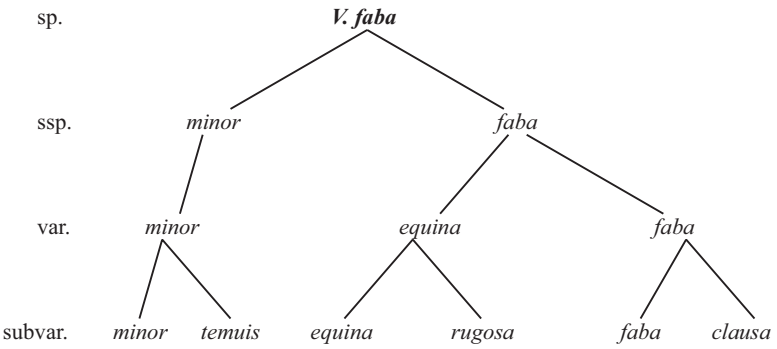

Fig. 7.5 Taxonomy of *Vicia faba* as suggested by Hanelt (1972)

polymorphism (AFLP) genotype data: (a) Asian faba bean genetic resources were divided into a group, and significantly differentiated from genetic groups of Europe and North Africa (Zeid et al. 2003); (b) the faba bean genetic resources from the rest of the world were significantly different to that from China, and the genetic resources of faba bean collected from the spring sowing area of China were significantly different to those from the winter sowing area of China (Zong et al. 2009). In addition, SNP markers were also used to study the genetic diversity within and between faba bean populations, which could distinguish Australian faba bean genetic resources from different geographical sources (Kaur et al. 2014a, b). To date, however, molecular markers have been unable to distinguish populations of different seed sizes (Gol et al. 2017).

7.1.2 Agronomic and Market Important Traits

The size and shape of dried faba bean seeds (Fig. 7.6) varies greatly, with the smallest grain weight 20 g and the largest of more than 200 g. The seed coat may be white, milky white, light green, green, brown, red or purple, and the color of the umbilicus is divided either light gray or black. The number of seeds per pod of faba beans varies from 2–8, and the maximum length of fresh pod can reach 35 cm (Fig. 7.7).

The fresh pods of faba bean exhibit different postures on the stem: uplifted, outspread or drooping (Fig. 7.8). The uplifted plant type is suitable for small pod and small grain varieties of dry faba bean. The outspread pod posture plant type is suitable for large pod and large grain faba bean varieties for vegetable purpose. The drooping pod posture plant type is suitable for the long pod type of faba bean for vegetable purpose. Dry pods of faba bean can be divided into two pod types: semi-soft or hard, according to pod shell hardness (Fig. 7.9). Semi-soft pods are hard to crack when they are dry and ripe, and are often used as an important targeted agronomic trait in faba bean breeding to adapt for mechanized harvesting by reducing the grain loss in the process of harvest.

Fig. 7.6 Dry grain of faba bean. (Photo by Zong Xuxiao)

Fig. 7.7 Long pods of faba bean. (Photo by Zong Xuxiao)

Fig. 7.8 Fresh pod postures on the stem. (Photo by Bao Shiying and He Yuhua)

Fig. 7.9 Pod shell hardness (left: semi-soft pod; right: hard pod). (Photo by Bao Shiying and He Yuhua)

7.1.3 Economic Importance

Faba bean is a leguminous crop rich in protein and well adapt to the arable land under most of the world's climatic conditions. It is widely used for feed and food and is generally considered to have good nutritional value (Katell et al. 2010). Faba bean can be divided into four quality types according to the presence or absence of tannin in the testa and the content of vicine (V) and covcine (C) in the cotyledon. The dietary nutritional value of faba bean varieties depends on the content of tannin, vicine and covicine (VC). Low tannin content usually leads to higher protein and energy digestibility in monogastric animals, and low VC content has a positive impact on the production performance of laying hens and broiler chickens (Katell et al. 2010). In addition to the positive effect of using no-tannin cultivars in single-stomach animal feed, the development of faba bean cultivars with very low VC content has real advantages in the nutritional performance of poultry feed and for the food safety of humans (Katell et al. 2010). The dry grain, fresh seeds and sprouts of faba bean are very nutritional food sources for the human diet, according to analyses by the Institute of Health, Chinese Academy of Medical Sciences (IHCAMS 1981), Table 7.1.

Dry faba bean grain in most producing countries outside China is mainly used for export and for animal feed; and in the traditional faba bean production countries like Egypt, dry faba bean is used as human food. At present, faba bean growers in China mainly focus on the production of fresh pods and the supply of legumes for vegetables. Fresh faba bean seeds after peeling are used in Chinese cuisine. Dried faba bean seeds are mainly used for processing spicy faba bean paste and snack food. Dried fresh stems and leaves of faba beans are good fodder for cattle, sheep and pigs. In addition, faba bean flowers contain a large amount of L-dopa (7% of the

Table 7.1 Nutritional composition of faba bean (per 100 g)

Items	Dry faba bean	Fresh faba bean	Faba bean sprouts
Water (g)	13.0	77.1	63.8
Protein (g)	28.2	9.0	13.0
Lipid (g)	0.8	0.7	0.8
Carbohydrate (g)	48.6	11.7	19.6
Caloric value (kcal)	314.0	89.0	138.0
Crude fiber (g)	6.7	0.3	0.6
Ash (g)	2.7	1.2	2.2
Calcium (mg)	71.0	15.0	109.0
Phosphorus (mg)	340.0	217.0	382.0
Iron (mg)	7.0	1.7	8.2
Carotene (mg)	0	0.15	0.03
V_{B1} (mg)	0.39	0.33	0.17
V_{B2} (mg)	0.27	0.18	0.14
V_{pp} (mg)	2.6	2.9	2.0
V_C (mg)	0	12.0	7.0

dry base), which can be used to make faba bean flower tea, which has purported dietary and therapeutic effects on Parkinson's disease and Alzheimer's disease in the elderly. Increasing demand for fresh faba bean consumption in China, India and other Asian countries has led to greater economic importance of the faba bean industry in those countries. This sets an example for other faba bean producing countries of more diversified usage of faba bean and better consumption of dry and fresh grains of faba bean around the world.

7.1.4 Ecologic Importance

The faba bean has a conical root system; the main root is strong and can reach down 1 m in depth, bringing to the upper soil layer nutrients, especially calcium and phosphorus. There are many lateral roots on the superior meristem of the main root, which are horizontally distributed on the soil surface and extend up to 50–80 cm before descending. Most of the faba bean root system is concentrated in the upper 30 cm of soil. There are many nodules clustered on the main root and lateral root, generally near the surface with a few nodules below 30 cm (Fig. 7.10). Nodules on the main root are large. The development of root nodules is closely related to the

Fig. 7.10 Root system and nodules of faba bean. (**a**) faba bean plants, (**b**) faba bean roots and rhizobia nodules. (Photos by Zong Xuxiao)

growth and yield of faba bean. The rhizobium of *Vicia faba* are associated with other plants of the Fabaceae such as pea, lentil and vetch, and can be inoculated using their rhizobium.

Faba bean is conducive to the healthy development of agricultural ecosystems, such as the renewable input of nitrogen to crops and soil through biological nitrogen fixation, and it benefits the diversification of planting systems. Seasonal fluctuations in the grain yield of faba beans and their gradual integration into traditional farming systems have reduced the importance of the application of nitrogen fertilizer by allowing faba beans to use their robust biological nitrogen-fixation capacity to maintain soil N levels, while cereal based farming systems rely heavily on fossil fuels (N fertilizers, heavy mechanization). Previous studies of faba beans in cropping systems have tended to focus on its occasional use as a spacing crop in intensive rotation of major cereals, but lacked information on the effects of the previous crops on faba beans. *Vicia faba* has the greatest capacity of biological nitrogen fixation among the cold season crops, and its growth and development relies heavily on its nitrogen fixation and adds to the soil a large amount of nitrogen for the rotation crops. As a result, the nitrogen fixation of faba bean maximizes the yield of its subsequent rotation crops, and those benefits are often very high. Several studies have shown significant nitrogen conservation effects (up to 100–200 kg of nitrogen per ha). However, it is necessary to assess the potential risks to the plant-soil systems associated with faba bean cultivation through nitrate leaching or N_2O emissions into the atmosphere as a result of the rapid mineralization of nitrogen-rich residues. It is important to develop improved precautionary measures, such as cash crops, intercropping or no-till techniques, and to provide farmers with rational advice to minimize the adverse environmental impacts of incorporating faba beans into their cropping systems. The extent to which current faba bean yield are changing and the reasons for their reduction need to be studied. Faba beans are likely to be a key component of future farming systems. This will help to address the growing demands for agriculture by consumers and governments to reduce the environmental and climate impacts on agriculture through new and more sustainable ways of producing quality food.

7.1.5 Breeding for Sustainable Agriculture

Faba bean plays a key role in crop rotation systems by reducing energy costs, improving the physical conditions of soil, and reducing pest and weed populations. Despite these advantages, since the 1960s, there has been a general trend of declining hectarage for dried faba bean production globally. At the same time, industrialized, largely cereal-based systems that rely heavily on fossil fuels are replacing traditional agricultural systems (Diego 2010). However, given the limits of fossil energy and a renewed focus on health and the environment, it is time to reassess the potential role of legumes, such as *Vicia faba*, *the king of nitrogen fixation*, as a nitrogen source for future farming systems (Jensen et al. 2010;

Köpke and Nemecek 2010). Faba bean plays an important role in the biosphere and the available nitrogen cycle (Diego 2010), notwithstanding its concomitant low and unstable yields, and susceptibility to biotic (Sillero et al. 2010) and abiotic stresses (Khan et al. 2010; Link et al. 2010). To facilitate wider adoption, faba beans should be improved to make them more attractive to both producers and consumers (Diego 2010). This can be achieved by: (a) adjusting planting methods (Jensen et al. 2010); (b) improvement of integrated pest management strategies (Pérez-de-Luque et al. 2010; Stoddard et al. 2010); (c) creation of major disease resistant genotypes (Sillero et al. 2010) through the development of abiotic stress tolerant genotypes such as overwintering frost (Link et al. 2010) and drought (Khan et al. 2010); (d) genotype development through improved adaptation to environmental change (Patrick and Stoddard 2010) and (e) reduction of the tannin and vicine – convicine contents to improve nutritional quality (Crépon et al. 2010).

In the extensive faba bean germplasm resources preserved, all the traits of interest have significant genetic variation, providing a good resource for faba bean breeding (Duc et al. 2010). Rapid and reliable screening methods have been applied to meet the needs for fungal disease resistance and tolerance breeding programs (Sillero et al. 2006; Tivoli et al. 2006), parasitic weeds (Rubiales et al. 2006) and abiotic stresses (Stoddard et al. 2006). Many of these interesting traits have been incorporated into modern cultivars, but others, many of which are quantitatively controlled by multiple genes, are more difficult to manipulate. Successful application of biotechnology to *Vicia faba* resistance breeding will require reliable biological knowledge of *V. faba* and potential resistance mechanisms. Although achievements have been made in tissue culture and gene transformation, faba beans still lag far behind other crops in biotechnology. Similarly, although important QTLs (quantitative trait loci) studies have been identified (Torres et al. 2006, 2010), they are still insufficient for effective application of marker-assisted breeding (Diego 2010). It is assumed that the genomic region of QTLs in genetic linkage maps have a limited degree of saturation, making it difficult to identify the most closely-connected markers and to determine the exact location of QTLs (Yang et al. 2019). With new improvements in marker technology and the integration of comparative localization and functional genomics, breeding efficiency may soon be improved (Dita et al. 2006; Rispail et al. 2010).

7.2 Cultivation and Traditional Breeding

7.2.1 Current Cultivation Practices

Faba bean can tolerate slightly heavier soils than peas, which makes them more versatile for cropping in a range of arable land conditions. The seed is well able to withstand prolonged cool conditions before germination; however, faba bean is

susceptible to drought stress, but early establishment of the spring crop allows development of an extensive root system, which helps to withstand dry conditions. Generally, autumn-sown cultivars, known as winter beans, can be planted in late autumn on heavier and more moisture-retentive soils to allow establishment of the root system before winter. A period of low temperature over winter encourages the production of multiple shoot branches which are compact in early spring until they elongate as temperature rises. Winter beans are planted at a lower density than the spring cultivars to allow for the increased number of stems, all of which are productive. Overwintering of autumn-sown beans effectively makes them more susceptible to foliar diseases caused by fungal pathogens such as *Botrytis fabae* and *Ascochyta fabae* but modern cultivars are much more tolerant of these fungi. Fungal pathogens can also be managed by chemical treatment during the growing season (Anthony 2017).

Due to the wide planting area of faba beans in China, and the differences in climate, soil, topography, socioeconomic development level and farming systems, a range of traditional planting methods have evolved. The typical faba bean-based intercropping system can be summarized as follows:

(a) Faba bean intercropping with wheat. The yield of faba bean and wheat intercropping is 20–30% higher than that of wheat monocropping. In the intercropping of faba bean and wheat, if faba bean is the main crop, 3 rows of faba beans and 1 row of wheat are used; If wheat is the main crop, 1 row of beans and 2–8 rows of wheat are used; The practice of planting half faba bean and half wheat, each of two rows, can be also found.
(b) Faba bean intercropping with maize and sweet potatoes. In the northwest, Yangtze River basin and southern provinces of China, this practice is widely used. Generally, the beds are 1.7–2 m wide, 1–2 rows of faba beans are planted on both sides of the beds, or 2–3 rows of faba beans are planted in the middle of the beds, and maize is sown or transplanted in early April of the next year. The symbiotic period of the two crops is 50–60 days. At the end of May, faba beans are harvested, and then sweet potatoes are planted between rows of maize, thus forming a triple cropping pattern of faba beans, maize and sweet potatoes.
(c) Paddy stubble zero tillage faba bean. In the autumn sowing area, rice stubble follows faba bean with no-tillage cultivation (Fig. 7.11), using the moisture remaining in the soil after rice. At the beginning of the ripening of rice, the faba beans are pressed into the soil at the side of rice roots, trenching and then stripping out of extra moisture (1.5–2.5 m wide seed bed, with a side channel 30–50 cm deep) for the field water supply and drainage conditions, to a good ensure faba bean seedling rate at emergence (Fig. 7.12).
(d) Faba bean for two harvests. Faba bean has strong reproductive ability. In the region along the Yangtze River in Jiangsu province, appropriate faba bean cultivars with early seeding time are made full use of in late autumn and the winter season. Branches are cut for animal feed after 60 days' growth, then branches are harvested again for feeding purpose, and again the following year after fresh pods are collected for vegetable purpose. Due to the early sowing of faba bean,

Fig. 7.11 Rice stubble followed by faba bean with no-tillage cultivation. (Photo by Zong Xuxiao)

Fig. 7.12 Rice-faba bean no-tillage cultivation on 1.5–2.5 m wide seed bed with side channel of 30–50 cm deep. (Photo by Zong Xuxiao)

a strong root system forms before winter, and a large number of regenerative branches grow after mowing before winter, so the fresh faba bean yield is 5–10% higher than that of normal sowing practice. This technique has good dissemination in Jiangsu and Zhejiang provinces, and the central and southwestern parts of China.

7.2.2 Current Agricultural Problems and Challenges

The current agricultural problems and challenges of faba bean in China and elsewhere can be summarized as follows from Bao et al. (2016):

(a) As a minor crop, there are no specific plans for seed industry development for faba bean. Faba bean industrial was developed by local governments or farmers in accordance with the market, and their own will to develop strong adapted, quality seed sources is difficult to guarantee.
(b) Due to large seed size, faba bean seed production cost and farmers' planting cost are high. There is no relevant supportive policy for the development of faba bean seed industry and subsidy measures for the breeding of faba bean cultivars. Seed enterprises are unwilling to develop the faba bean seed industry, which leads to low overall supply rate of fine quality faba bean cultivars.
(c) There is no stable faba bean seed base in all faba bean production areas and regions. Given the natural high variation of the original populations of heterozygous crops, new cultivars of faba bean degenerate rapidly. There is no relatively isolated and stable faba bean seed base, so that the natural degradation of cultivars is fast and seed purity is quite low.
(d) Mechanized seeding, field management and harvest together are the main restricting factors affecting industrial development of faba beans worldwide due to the large seed size and flat irregular shape.
(e) Lack of cultivars for commercial vegetable faba bean production globally. The commercial vegetable faba bean type should have long pods, super large seeds, a soft seed coat, sweet taste and a soft or crisp mouthfeel. Also, the nutritional value should also be optimized.
(f) Lack of promotion of faba bean foods in the super markets. The food industry should follow up with needs for faba bean consumption globally, in developing and developed countries, especially for fresh faba bean based food.

7.2.3 Improvement of Strategies

The faba bean industry is generally weak in most producing countries; improving it is a shared challenge. For fast and healthy development of faba bean industry, Bao et al. (2016) suggest the following strategies:

(a) Integration and development of faba beans with other compatible crop industries. Faba beans have a triple nourishment function for people, livestock and soil. It is necessary to give full play to the advantages of faba beans and integrate them effectively into agricultural production systems. Faba bean occupies a certain key position in the rotation system, including as green manure. Faba bean needs to be integrated into the vegetable industry, and develop in the direction of its acceptance as a high-quality nutritional and healthy diet vegetable, as well as integrated with the forage industry and developed to produce a high quality forage.
(b) In-depth integration of primary, secondary and tertiary industries. Faba bean has an advantage in natural snack food processing, so it is necessary to extend the industrial chain longitudinally. Horizontal expansion of its application fields can improve the diversified industrial system and constantly enhance its value.

(c) Developing light simplified field cultivation methods of faba bean. There is a relatively large labor input in the production of faba beans. Constant change should be made in the mode of production, select and breed new faba bean cultivars suitable for mechanization and to develop machinery and equipment suitable for different production modes of faba beans, promote the in-depth integration of agricultural machinery and agronomy, improve the level of mechanized production, improve production efficiency and reduce production costs.
(d) In top-level design, policy formulation should fully recognize the importance of faba beans in the national economy, both as a fertilizer to improve soil and ensure the effective supply of regional food processing, and as a vegetable as well as a high quality forage grass.
(e) While developing staple crops, it is necessary to give consideration to the development of minor crops, paying more attention to the combination of land use and cultivation, appropriately increasing the planting proportion of faba beans and other crops in the cultivation of farmland, and establish reasonable long-term rotation mechanisms. There should be clear scientific and reasonable crop layout planning, and logical policies and measures for long-term combination of land use and cultivation and sustainable development of agriculture.

7.2.4 Traditional Breeding Methodologies and Limitations

High levels of genotype environmental interaction (G × E) have been demonstrated in *Vicia faba*, particularly in contrast to germplasm pools and across broad environments. Within a germplasm bank, there are also differences in the degree of environmental adaptation among different cultivars. For example, in China, when the winter is long and severe, the cultivars sown in autumn have strong adaptability in winter, and the cultivars sown in spring have strong adaptability in spring. To a large extent, this change can be attributed to the optimal phenological changes in different geographical and climatic regions, but the changes in stress between regions (e.g. dominant diseases) are also important (Duc et al. 2015). Trials in Germany and by ICARDA have shown that central European cultivars produce very low yields in Syria, while Mediterranean cultivars produce well in both environments and bloom early (Kittlitz et al. 1993). So, universally-adapted cultivars of faba bean are widely acceptable, although they are difficult to create by traditional breeding methodologies such as single plant selection and crossbreeding of cultivars according to breeders' experience.

7.2.5 Mutation Breeding

Faba beans are reproductively isolated from all other *Vicia* species. This limits the usefulness of existing germplasm collection, new collection tasks or new methods for generating genetic diversity or genetic variation in faba bean improvement. The

two main modern methods for introducing new mutations into faba beans are mutation and genetic transformation. Sjodin (1971) experimented with a series of mutagens, including X-rays, and produced a large number of mutant phenotypes, most of which were determined by recessive alleles targeting the wild allele. Oldach (2011) listed 19 mutant faba bean varieties from 1959–2009. The mutant traits included early maturity, plant structure, yield, dwarfing, protein content, disease resistance and lodging resistance. The main character of mutagenesis is the determination or terminal inflorescence growth habit restricted by the recessive allele of *ti* gene (Duc et al. 2015). Even so, the traditional breeding methodologies have limited the achievement of more and better cultivars for optimized agronomy in different major production areas.

7.3 Germplasm Diversity and Conservation

Germplasm, or genetic resources, refer to the genetic material passed from the parent generation to the offspring generation. For example, landrace, varieties, important genetic materials and wild relatives are all within the scope of germplasm resources (Haussmann et al. 2004).

7.3.1 Germplasm Diversity

According to statistical data, there are 38,360 faba bean accessions conserved in 43 countries and within the CGIAR system (Table 7.2). ICARDA safeguards the largest collection in the world with accessions from 71 countries with a high percentage of unique accessions (Duc et al. 2015). China has collected 5900 faba bean accessions, made up of 65% native and 35% foreign accessions.

Considerable progress has been made on genetic diversity of faba bean. Descriptors and data standards have been published for the criteria on standard evaluation of morphological and agronomic characters (Zong et al. 2006). According to eight morphological traits such as fertility, plant height, stem flowering, the lowest pod, the highest pod, pod number per plant, grain number per plant and yield, 106 faba bean cultivars from Ethiopia and Afghanistan were used for genetic diversity analysis. Results showed that plant height and yield of different source materials have obvious differences (Polignano et al. 1993). Link et al. (1995) carried out genetic analysis with random amplified polymorphic DNA (RAPD) markers on 13 European small-grain, 6 European large-grain and 9 Mediterranean varieties and found that genetic diversity within the small-grain varieties was relatively high. Terzopoulos et al. (2003) conducted statistical data analysis on 15 morphological traits and 7 yield related traits of 55 landrace accessions in Greece, and found that small-grain faba bean was clustered into one group and Mediterranean faba bean were divided into four subgroups. Furthermore, Terzopoulos and Bebeli (2008)

Table 7.2 Major world *Vicia faba* genetic resource collections in 2014

Country	Institute/city	Number of accessions
Syria	ICARDA/Aleppo	10,045
China	CAAS/Beijing	5900
Australia	Australian Grain Gene Bank/Victoria	2445
Germany	Gene Bank IPK/Gatersleben	1920
France	INRA/Dijon	1900
Russia	VIR/St Petersburg	1881
Italy	Genebank/Bari	1876
Morocco	INRA/Rabat	1715
Spain	CNR/Madrid	1622
Poland	IOPG-PA/Poznan	1258
Ethiopia	PGRC/Addis Ababa	1118
Spain	IFAPA/Cordoba	1091
Poland	PBAI/Radzikow	856
Portugal	INRB—IP/Oeiras	788
USA	USDA/Pullman	750
The Netherlands	DLO/Wageningen	726
Bulgaria	IIPGR/Sadovo	692
World total	More than 43 known collections	38,360

analyzed genetic diversity of landrace accessions in Greece through ISSR markers, and found that the pellet types still aggregate into one group, and the Mediterranean *Vicia faba* can be divided into at least two groups. Zeid et al. (2003) analyzed 79 faba bean varieties from Asia, Europe and North Africa with 8 AFLPs, and amplified 477 bands. Further studies have found that cultivars from Asian sources can be grouped into one category, while those from other places have no obvious classification. Zong et al. (2009) used 10 AFLP tags to analyze 243 *Vicia faba* resources (including 39 foreign winter-sowing type accessions, 201 domestic winter sowing type accessions and 3 spring sowing type accessions), and obtained 266 polymorphic bands. Significant differences were found between Chinese and foreign resources, and between spring sowing type and winter sowing type accessions. Zong et al. (2010) used 12 AFLP markers to analyze genetic diversity of 39 domestic spring-sowing type resources, 136 overseas spring-sowing type resources (from Africa, Asia, Europe and Canada) and 41 breeding materials (from ICARDA), and found that there were obvious differences in spring-sowing type resources with geographical distribution, which showed that the Chinese and foreign resources differ significantly. In addition, ICARDA's genetic resources exhibited relatively low diversity in breeding materials.

Wang et al. (2012) used 802 faba bean resources from different regions of the world and 11 ISSR markers to obtain 209 bands with polymorphism, and found that the diversity of resources from Central China was the lowest. Moreover, obvious differences between spring-sowing resources and winter-sowing resources in China again were found. The resources from Zhejiang, Sichuan and Guizhou are quite different from those from other provinces. Furthermore, China's resources are

genetically distinct from those of other countries. Gong et al. (2011) analyzed the diversity of 29 *Vicia faba* resources in China and Europe with 11 EST-SSR markers, and found that the genetic diversity of Chinese resources was relatively low, and advised increasing the introduction of foreign resources. In Turkey, 25 polymorphic SSR markers were used to investigate the genetic variation in 22 faba bean genotypes (from ICARDA and Turkey); sufficient genetic diversity among the tested faba bean genotypes (especially those cultivated in Turkey) was observed and could be used in faba bean breeding programs (Tufan and Erdogan 2017).

Kaur et al. (2014a, b) used 768 SNP markers to evaluate 45 faba bean accessions, among which 657 SNP markers were used to obtain bands with polymorphisms. This study found that these beans materials were mainly divided into two major categories (G-I and G- II), and the G-II can be divided into three types (A, B, C). Backouchi et al. (2015) analyzed the levels of polymorphism across 12 Tunisian populations, 3 major and 9 minor from different locations, with morphological traits and RAPD markers. Results showed that the Takelsa population exhibited the highest Nei and Shannon indices, indicating this population was the most heterogeneous, which is ideal for breeding programs). Göl et al. (2017) studied 255 faba bean germplasm accessions with the help of 32 SSR markers. All materials were divided into two categories according to the neighborhood connection algorithm ($r = 0.91$), and were clustered according to geographical sources and seed size. Population structure was also determined and agreed with the dendrogram analysis in splitting the accessions into two subpopulations. Furthermore, El-Esawi (2017) used SSR markers to evaluate diversity and structure of 35 faba bean genotypes originating from North Africa, East Africa, and the Near East. Structural analysis and cluster analysis revealed that the 35 faba bean genotypes may be assigned to two populations.

7.3.2 Germplasm Conservation

The in situ conservation and evaluation of any plant species depend on its reproductive system. Faba bean is partially cross-pollinated by insects. The reproductive system in the dominant population follows a pattern of interbreeding. The cross-pollination rate varies greatly depending on genotype and geographical location; pollination mainly depends on climatic factors and insect pollinators in the field. Most of the experimental data on the gene flow of faba bean indicate that the conservation and reproduction of faba bean genetic resources must exclude the involvement of insect pollinators. Using insect-proof cages is an effective way to conserve *Vicia faba* genetic resources. When a great number of faba bean genetic resources need to be multiplied and the quantity of each seed sample is often very large, the insect-proof cage is no longer applicable, because of high cost, difficult operation and management, and will also lead to a decline of yield caused by inbreeding recession. The development of germplasm conservation techniques of faba bean depends on maintaining appropriate gene flow between different faba bean

accessions during multiplication practices in the field; appropriate isolation crops are needed. According to our field tests in faba bean breeding, in order to reduce gene flow between different accessions or genotypes, the use of a 3-meter isolation distance and biological barrier (rapeseed or rye) can reduce the hybridization between adjacent accessions by more than 95%. For large numbers of faba beans to be planted, the field must be kept at least 50–100 m away from other faba bean fields to maintain seed purity (Bao et al. 2016).

The genetic resources of ex situ collections of faba bean are mainly local landraces and mass selections from landraces, open-pollinated populations, synthetics, inbred lines and hybrids (Duc et al. 2010). The main practice for long-term conservation of ex situ collections is to preserve the original dry seeds and their purified dry seeds in cryogenic and ultra-cryogenic cold storage with different specifications and construction forms (Bao et al. 2016). Faba bean genetic resources are usually conserved in gene banks at −20 °C to −18 °C for long-term strategic safe storage (Zheng et al. 1997). For the medium-term repository, a storage temperature of around 5 °Cis used by the National Genbank of the CAAS (Chinese Academy of Agricultural Sciences, Beijing, China), USDA and ICARDA (Bao et al. 2016).

7.4 Molecular Breeding

7.4.1 Genetic Linkage and QTL Mapping

The role of biotechnology in faba bean breeding can be applied through maker-assisted selection in breeding programs. This relies on breakthrough in-depth studies on faba bean germplasm, high density SSR/SNP based genetic linkage map construction and important traits (QTL) analysis. The construction of genetic linkage maps is an important aspect of genetic research. Until now, most genetic linkage maps of faba bean were based on molecular markers and limited to isozymes, RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism) and ITAP (intron targeted amplified polymorphic markers) in the world. The genetic linkage maps of faba bean constructed with SSR (simple sequence repeats) and SNP (single nucleotide polymorphisms) markers are very limited (Ma et al. 2013; Webb et al. 2015). Van de Ven et al. (1991) constructed the first genetic linkage map of faba bean containing markers such as morphological markers, isozyme markers, RFLP and RAPD, which included 17 markers located on 7 linkage groups. Torres et al. (1993) used Vf6 × Vf35 F_2 and Vf6 × Vf173 F_2 populations with isozyme, RFLP, RAPD markers to build a map with 11 linkage groups. Satovic et al. (1996) constructed a linkage map, which included 157 markers (1 morphological trait, 9 isozymes, 147 RAPD) and 48 linkage groups, 6 of which were distributed on specific chromosomes and covered about 850 cM of the faba bean genome. Patto et al. (1999) built a faba bean integrated genetic map by using Vf6 × Vf27 F_2 population and 116 markers containing 1 morphological character, 7 isozymes, 105 RAPD and 3 grain protein gene.

Orobanche crenata F. (broomrape) is a root parasitic plant, and can devastate completely faba bean crop production all along the Mediterranean Coast; it is necessary to cultivate resistant faba bean cultivars to overcome this problem. Roman et al. (2002) constructed a linkage map with 16 linkage group and used Vf6 (sensitive) × Vf136 (resistance) F_2 population, with 121 markers containing 117 RAPD, 2 isozymes, and grain protein gene. They detected 3 QTLs related to *Orobanche* resistant, namely *Oc1*, *Oc2* and *Oc3*, showing phenotypic variation. Furthermore, Roman et al. (2004) constructed one integrated map with 192 markers including 2 morphological markers, 6 isozymes, 3 grain protein genes, 176 RAPD and 4 SSR markers, and a total length of 1559 cM; the mean distance between markers was 8 cM. Diaz-Ruiz et al. (2009a, b) tested Vf6 × Vf136 RIL populations to study hairy broomrape resistant QTLs. They found two resistant loci *Of1* and *Of2*, one located in the chromosome I explaining 7% of the phenotypic variation, and the other one located in the chromosome III explaining 9% of the phenotypic variation. Diaz-Ruiz et al. (2010) used the same Vf6 × Vf136 RIL populations with 165 individuals to build a map containing 277 markers (238 RAPDs, 4 isozymes, 5 ESTs, 1 SCAR, 6 SSRs, 2 STS, 21 ITAPs). As a result, the map containing 21 linkage group and 2856.7 cM. Subsequently, QTL mapping detected 4 positive genes, in which *Oc2* and *Oc3* were consistent with previous studies, and 2 new environment-dependent loci *Oc4* and *Oc5* were identified. Gutierrez et al. (2013) used a 29H × Vf136 RIL population with 165 individuals. As a result, the map containing 172 markers, 29 linkage groups and 1402.1 cM. Subsequently, QTL mapping was carried out and found 7 QTL loci, *oc7–oc13*.

Ascochyta fabae Speg. blight is an important fungal disease. Roman et al. (2003) used Vf6 (resistance) x Vf136 (sensitive) F_2 populations with 196 individuals. As a result, the map contained 121 markers, 16 linkage groups and 1445.5 cM. Two resistance QTLs, *Af1* and *Af2*, were identified by QTL mapping, which accounted for 46% of phenotypic variation. Avila et al. (2004) used 29H × Vf136 F_2 populations to construct linkage map including 103 markers, 18 linkage groups and a total length of 1308 cM. After QTL mapping, 6 positive loci were found *Af3–Af8*. Among them, *Af3*, *Af4*, *Af5* and *Af7* were correlated with disease-resistant phenotypes of stems and leaves, while *Af6* was correlated with disease-resistant phenotypes of leaves and *Af8* was correlated with disease-resistant phenotypes of stems. Diaz-Ruiz et al. (2009a, b) constructed a genetic linkage map with 277 markers and 21 linkage groups covering 2856.7 cM of the genome. *Af1* and *Af2* were associated with resistance to *Ascochyta* blight, including *Af1* located in chromosome III and *Af2* located on chromosome II. In total, they explained 24% and 16% of the phenotypic variation of leaf and stem, respectively.

Arbaoui et al. (2008) used a F_6 RIL population to build genetic maps and QTL mapping. As a result, the map contained 132 markers and 21 linkage map covering 1635.39 cM of the genome. Five QTLs related to frost resistance and three sites related to fatty acid content were detected. Ellwood et al. (2008) used Vf6 × Vf27 RIL populations containing 94 individuals. As a result, they constructed a genetic linkage map containing 127 ITAP markers and 12 linkage groups covering 1685.8 cM of the faba bean genome. Cruz-Izquierdo et al. (2012) used Vf6 × Vf27 RIL populations

containing 124 individuals and constructed a genetic linkage map covering 1875.1 cM of the faba bean genome with 258 markers (167 ITAP, 3 RGA, 11 SSR, 71 RAPD, 2 isozymes, 3 seed proteins, 1 morphological marker). Subsequently, QTL mapping was performed for flowering time, flowering length, pod length, number of single pod seeds and number of single pod ovules, and it was found that 12 QTLs could be detected for 2 years. Satovic et al. (2013) integrated different populations Vf6 × Vf27 RIL, Vf6 × Vf136 RIL, 29H × Vf136 RIL and 11 F_2. They constructed an integrated genetic linkage map containing 729 markers including 69 universal markers, covering 4602.0 cM of the faba bean genome, and the average genetic distance between the markers was 6 cM. Our group used 128 SSR markers to construct a new map based on Chinese cultivars. This map contained 15 linkage groups. The length was 1587 cM and the average distance between markers was 12.4 cM (Ma et al. 2013). Moreover, a new integrated linkage map contained 465 SSR loci distributed among 7 linkage groups and spanning a length of 4516.75 cM with an average distance 9.71 cM between adjacent loci were constructed this year (Yang et al. 2019). El-Rodeny et al. (2014) developed an integrated map with 552 markers, covering a total length of 684.7 cM in the genome. Webb et al. (2016) developed a new map containing 687 SNP markers which were placed on six linkage groups. Ocaña-Moral et al. (2017) developed a set of SNP markers by RNA-sequencing. 92 new SNP markers were combined with previous data set to obtain the most complete map of 2796.91 cM including 257 loci assembled into 19 LGs in the 29H × Vf136 faba bean population.

7.4.2 In Vitro Regeneration and Genetic Transformation

Successful application of biotechnology in plant breeding requires an efficient method of in vitro regeneration. So far, there have been only a few successful attempts related to regeneration and tissue culture of faba bean. The first reported stable *Vicia faba* germline transformation used in vitro regeneration of *Agrobacterium*-infiltrated (non-meristematic) internode stem segments (Böttinger et al. 2001). Another study reporting transgenic *V. faba* event was infiltrated excised (meristematic) embryo axes with *Agrobacterium* and successfully recovered stable transgenic lines (Hanafy et al. 2005). However, both methods have a low primary conversion efficiency and rely on a slow and highly manual process of micrografting a putative transgenic bud material onto a non-transgenic root. Despite extensive research since the 1960s, it is difficult to establish a reliable faba bean regeneration system (Duc et al. 2015).

The main obstacle to genetic transformation of *Vicia faba* is the lack of effective and stable plantlet regeneration systems. The most widely used method of transferring foreign genes into dicotyledons is *Agrobacterium*. However, the use of *Agrobacterium*-mediated transgenic method to produce transgenic plants based on stem segments, mature embryo discs and cotyledon nodes have been reported (Bottinger et al. 2001; Hanafy et al. 2005; Jelenic et al. 2000). The biolistic blast

gene delivery system was used to establish a regenerative and microprojectile-mediated transformation system. Although the main obstacles to transgenic faba bean plants appear to have been overcome, the number of transgenic plants introduced so far remains limited. Two successful studies used this method to improve protein quality. Gnanasambandam et al. (2012) and Hanafy et al. (2013) were the first to report evidence that *PR10a*, a gene-enhancing salt and/or drought tolerance in potato, was transferred into faba bean through the *A. tumefaciens*-mediated transformation system, which has the same effect in faba bean.

More recently, by preparation of gene cassettes for β-1, 3-glucanase from barley, chitinase from bean and cryIA (b) from *Bacillus thuringiensis* (BT), pBI-ChiBt and pBI-ChiGlu recombinant plasmid vectors (pBI121 based vector) were made and have been introduced into the *A. tumefaciens* strain LBA4404 that was subsequently used for faba bean transformation (Gorji et al. 2014). Results indicate that embryogenic calli are well suited as objective material for *Agrobacterium tumefaciens*-mediated transformation in faba bean. A total of 17 well-established shoots were transferred to new MLS medium including suitable antibiotics, of which 6 independent transgenic plants were successfully rooted on kanamycin containing selection media and then transferred to soil after 20 days (Gorji et al. 2014). Four plants out of the above mentioned 6 putative transgenic plants displayed the targeted end part of the *chit* transgene and *nos* terminator (Gorji et al. 2014). Subsequently, *bgn*13.1 and *cryIA (b)* genes sequences were amplified by PCR using specific primers from the 3 transgenic plants and the other 3 plants did not have these fragments (Gorji et al. 2014); this proved the successful construction of a relatively effective and reliable faba bean transgenic platform.

7.5 Conclusion and Prospects

7.5.1 An Overview of the Current Status

As a cool season legume crop, faba bean plays a critical role in improving cereal-based systems and soil fertility, and helps to develop sustainable cropping systems that involving faba bean, as it is preeminent in terms of biological nitrogen fixation efficiency. Breeding programs for resistance to abiotic stresses (early or late drought, heat, winter-hardy et al.) as well as to pests and diseases (broomrape, *Sitona* weevils, leaf miners, bruchids, pathogenic fungi) have made good progress at ICARDA and in European countries. In China, more than 110 faba bean cultivars were commercially registered at the national or provincial levels over the past 40 years. Since 2008, 43 new faba cultivars were registered (Table 7.3), almost all of them are large seeded type with 100-seed weight of over 120 g, with dual usage for dry and fresh seeds, except for the traditional dry grain cv. Zhijinxiaocandou from Guizhou province. The newly registered faba bean cultivars were bred mostly by traditional crossing methods and supplementary single plant selection.

Table 7.3 Faba bean cultivars registered in China since 2008

Cultivar name	Registered time (Y.M.D)	Provincial breeding program location
Lincan 6	2008.04.01	Gansu
Lincan 7	2009.03.27	Gansu
Lincan 8	2009.03.27	Gansu
Lincan 9	2011.03.04	Gansu
Lincan 10	2013.03.26	Gansu
Lincan 11	2015.04.13	Gansu
Lincan 12	2015.04.13	Gansu
Zhijinxiaocandou	2016.06.21	Guizhou
Jizhangcan 2	2009.12.29	Hebei
Ecandou 1	2015.10.26	Hubei
Sucan 1	2012.08.07	Jiangsu
Sucan 2	2012.08.07	Jiangsu
Tongcanxian 6	2016.06.07	Jiangsu
Tongcanxian 7	2012.08.07	Jiangsu
Tongcanxian 8	2016.06.07	Jiangsu
Tongcanxian 9	2012.08.07	Jiangsu
Qinghai 13	2009.12.10	Qinghai
Qingcan 14	2011.11.22	Qinghai
Qingcan 15	2014.02.07	Qinghai
Chenghu 15	2013.08.01	Sichuan
Chenghu 18	2009.07.01	Sichuan
Chenghu 19	2010.06.18	Sichuan
Chenghu 20	2014.07.31	Sichuan
Chenghu 21	2016.07.20	Sichuan
Yundou 9224	2008.01.10	Yunnan
Yundou 825	2009.01.16	Yunnan
Yundou 853	2009.12.16	Yunnan
Yundou 690	2012.09.29	Yunnan
Yundou 95	2012.08.27	Yunnan
Yundou 470	2014.06.09	Yunnan
Yundou 06	2016.01.18	Yunnan
Yundou 459	2016.12.15	Yunnan
Fengdou 22	2016.12.15	Yunnan
Fengdou 21	2016.12.15	Yunnan
Fengdou 20	2016.09.06	Yunnan
Fengdou 19	2016.09.06	Yunnan
Fengdou 18	2016.01.18	Yunnan
Fengdou 17	2014.06.09	Yunnan
Fengdou 16	2012.08.27	Yunnan
Fengdou 15	2011.11.09	Yunnan
Fengdou 14	2009.12.16	Yunnan
Fengdou 12	2011.03.15	Yunnan
Fengdou 11	2008.01.10	Yunnan

Although the dry faba bean production area was slightly reduced in the world (Fig. 7.1) and China (Fig. 7.2), the vegetable production of faba bean is increasing in the world (Fig. 7.13a) and in China (Fig. 7.13b) with very high yield potential (Fig. 7.13c) (FAOSTAT 2018), despite of that data are not available after 2008 on the FAO website. The market driven production of vegetable faba bean has increased in production area of China, and most of fresh pods of faba bean were provided to large cities like Shanghai, Nanjing, Hangzhou, Hong Kong, et al. at the price of over 1 USD per kg in March and April. This encouraged farmers to expand the sowing area for fresh pods production of faba bean, and breeders to pay more attention to vegetable cultivar breeding in China. According to statistics from the National Center for Extension of Agronomic Techniques, Ministry of Agriculture, China, the area of vegetable and faba bean cultivation in China expanded to 430,000 ha (6.42 million mu) in 2014 (Li et al. 2017). However, in 2005, the FAO database ceased inclusion of vegetable faba bean after 2005. The world total vegetable production area was only 0.20 million ha (Fig. 7.13a), and there were only 10,000–20,000 ha of vegetable production area in China at that time (Fig. 7.13a).

The fresh pod has become very popular in the local markets in China over the past 10 years (Fig. 7.14), as local residents and farmers became consumers of fresh faba beans. The rapid expansion of fresh faba bean sowing areas was mainly driven by large demand for fresh faba bean market. Vegetable faba bean took one-third of whole faba bean production area in 2014 in China, and now it occupies nearly half of the total area. Therefore, vegetable type is the right direction for faba bean industry in China, and probably for the whole world as well.

7.5.2 Current Research Initiatives and Recommendations for Future Research

Progress in faba bean genomics still lags behind other legume crops. Nevertheless, a wide range of genomic and post-genomic resources are being developed to boost genetic research and breeding applications. Different molecular marker sets, such as SSRs and SNPs, have been developed and used to construct more saturated linkage maps in order to identify genes or QTLs controlling major agronomic and stress related traits. Current efforts are focused on the development of highly accurate selective breeding tools, using NGS methods and RNA-seq technologies to refine the maps with functional markers. Moreover, translational genomics, based on the collinearity with model and related species, opens the possibility to identify candidate genes underlying agronomic important traits. Finally, a faba bean functional consensus map will be constructed to integrate all the previously-published genes and QTLs. The combination of these and new tools together with a close link between academic research and commercially-focused breeding programs may help researchers to find genes of interest and to speed the release of more competitive faba bean cultivars in the near future (Duc et al. 2015).

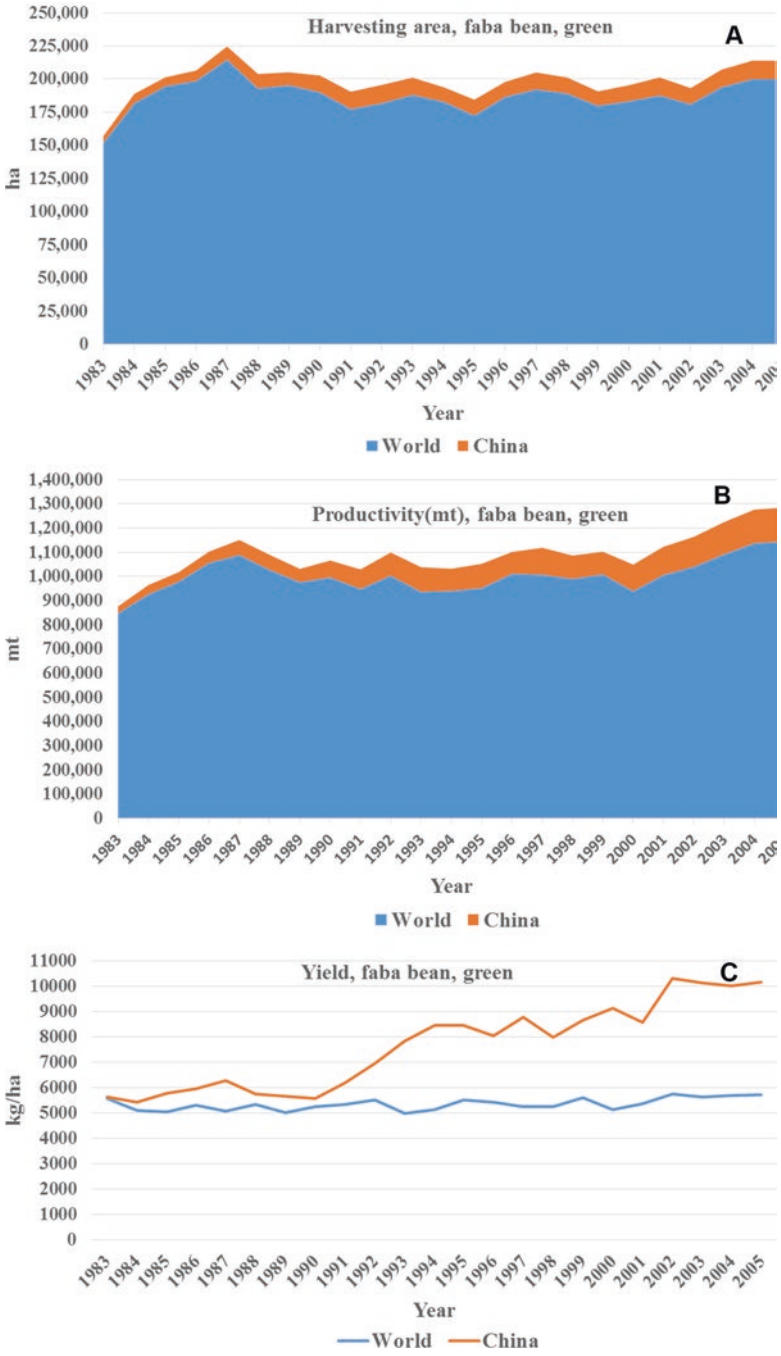

Fig. 7.13 Vegetable faba bean harvest area (**a**), productivity (**b**) and yield (**c**) in the world and China. (Source: FAOSTAT 2018)

Fig. 7.14 Fresh pods of faba bean selling in local market in Yunnan province, China. (**a**) Fresh faba bean pods in a free market, (**b**) Fresh faba bean seeds, (**c**) Fresh faba bean pods in a bag after harvesting, and (**d**) Fresh faba bean long pod type after harvesting. (Photo by Zong Xuxiao)

Currently, faba bean breeding has a large number of objectives to achieve, and the priorities depend on regional stress factors and market forces. As a result of climate change, stronger demands exist for genetic resistance to abiotic stresses (early or late drought, heat, frost, winter-hardy, waterlogging) as well as to pests and diseases (*Orobanche crenata*, *Sitona* weevils, leaf miners, bruchids and pathogenic fungi) in new geographic zones (Duc et al. 2015). Potential novel food uses of faba beans need to be addressed, as fresh vegetables, and also strengthened demands for low vicine and covicine, high protein and low tannin content genotypes. Inevitably, when the breeder needs to add more objectives to an existing program, either more resources have to be allocated to cover it or overall progress slows down. In addition

to large, characterized and structured genetic resources collections, rapid breeding methods and translational omics will help to streamline the breeding progress, so more of the objectives can be met. Considering the coming needs for the faba bean industry in the world, future breeding objectives of faba bean should include winter hardiness, heat tolerance, herbicide resistance, double-zero (low content of vicine and covicine), BNF (biological nitrogen fixation) efficiency, high photosynthetic efficiency, better flavor and palatability, dual usage both for forage grass and for green pods, as well as a favorable market price.

In the future, faba bean breeding would be more predictable than traditional breeding by genome editing technology, although it will heavily rely on in vitro regeneration systems. Once a stable and reliable in vitro regeneration system of faba bean achieves a breakthrough, the possibility of ideal phenotypic outcomes will be increased dramatically by CRISPR/Cas-induced gene or regulatory element rather than by natural or chemical/physical-induced variants such as EMS mutagenesis or radiation induced mutation.

Appendices

Appendix I: Research Institutes Relevant to Faba Bean

Institution	Specialization and research activities	Contact information and website
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences	Genetic resources and genomic studies, genetic improvement of faba bean, pea and other pulse crops	No. 12. Zhong Guan Cun South street, Haidian district, Beijing, China. http://ics.caas.cn/
Institute of Grain Crops, Yunnan Academy of Agricultural Sciences	Faba bean and pea breeding and agronomy	No. 2238, extension line, Beijing road, Panlong district, Kunming city, Yunnan province, China. http://www.ynicri.cn/
Institute of Crop Breeding and Cultivation, Qinghai Academy of Agriculture and Forestry	Faba bean breeding and agronomy	No.97, Ningzhang Road, Chengbei district, Xining city, Qinghai province, China. http://www.qhnky.com/
Institute of Crops, Sichuan Academy of Agricultural Sciences	Faba bean and pea breeding and agronomy	No.20, Jingju Temple Road, Jinjiang District, Chengdu city, Sichuan province, China. http://www.chinawestagr.com/zwyjs/

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Institution	Specialization and research activities	Contact information and website
Nantong Institute of Agriculture, Jiangsu	Faba bean breeding and agronomy	Yanjiang Road, Changjiang Town, Rugao city, Nantong city, Jiangsu province, China. http://yj.jaas.ac.cn/
Linxia Institute of Agriculture, Gansu	Faba bean breeding and agronomy	Hongyuan Xincun road, Linxia city, Linxia Hui Autonomous Prefecture, Gansu province, China
Australian Temperate Field Crops Collection – pulses and oilseeds	National Plant Genetic Resource Centres Vicia (vetches and faba bean)	Department of Primary Industries, Victoria. http://www.pulseaus.com.au/
Genebank, ICARDA	Faba bean genetic resources	ICARDA, Egypt. http://www.icrisat.org
International Center for Agricultural Research in the Dry Areas (ICARDA)	Faba bean breeding	ICARDA, Beirut, Lebanon. http://www.icrisat.org
Virology Laboratory, ICARDA	Virus Diseases of Food Legume Crops in WANA Region (Detection & Control)	ICARDA, Lebanon. http://www.icrisat.org
Department of Primary Industries, Biosciences Research Division, Grains Innovation Park	Faba bean genetic resources and breeding	Private Bag 260, Horsham, Victoria 3401, Australia. http://www.pir.sa.gov.au/home
Department of Plant Breeding, Spanish National Research Council	Faba bean genetic resources and breeding	Apdo. 3092, Córdoba E-14080, Spain. http://www.csic.es/
Sydney Institute of Agriculture, The University of Sydney	Faba bean breeding and agronomy	Sydney, Australia. https://sydney.edu.au/agriculture/
Tamworth Agricultural Institute, New South Wales Department of Primary Industries	Research on viral diseases of faba bean in Australia's Northern Grain Region	RMB 944, Tamworth 2340, Australia. https://www.dpi.nsw.gov.au/
Department of Primary Industries, Biosciences Research Division, VABC	Faba bean researches	1 Park Drive, Bundoora, Victoria 3083, Australia
Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes	Rhizobium-legume symbiosis, faba bean	Toulouse, France. http://www.ara.inra.fr/en
INRA, Research Unit – Genetics & Ecophysiology of Grain Legumes (UR-LEG)	Faba bean breeding	Dijon, France. http://www.ara.inra.fr/en
John Innes Centre	Faba bean research and breeding	Norwich, UK. https://www.jic.ac.uk/
School of Agriculture, Policy and Development, University of Reading	Faba bean research and breeding	Whiteknights, UK. http://www.reading.ac.uk/

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Institution	Specialization and research activities	Contact information and website
School of Agriculture, Food and Wine, The University of Adelaide	Faba bean research and breeding	Waite Campus, Glen Osmond, South Australia 5064, Australia. https://sciences.adelaide.edu.au/
School of Agriculture & Food Systems, Faculty of Land & Food Resources, University of Melbourne	Biotechnology to cropping and horticultural industries, including faba bean	Melbourne, Australia. https://research.unimelb.edu.au/
N.I.Vavilov Research Institute of Plant Industry	Faba bean genetic resources	St. Petersburg, Russia. http://vir.nw.ru/index.htm
Western Regional Plant Introduction Station, US Department of Agriculture-Agriculture Research service, Washington State University	Faba bean genetic resources and breeding	Pullman, Washington State, USA. https://www.ars.usda.gov/pacific-west-area/pullman-wa/
Grain Legume Genetics and Physiology Research Unit, US Department of Agriculture, Agricultural Research Service, Washington State University	Research on faba bean diseases, rhizobium in the US	Pullman, Washington State, USA. https://www.ars.usda.gov/pacific-west-area/pullman-wa/
Alberta Agriculture and Rural Development, Edmonton	Research on faba bean diseases, and breeding in Canada	Edmonton, Alberta, Canada. https://www.alberta.ca/ministry-agriculture-forestry.aspx

Appendix II: Faba Bean Genetic Resources

Cultivar	Important traits	Cultivation location
Qingcan 1	Seedlings erect, young stem light green. Main stem green, square. leaves upright, plant compact. Racemes purplish red, flag purplish red, veined pale brown, winged purple, with a black central disk, keel purplish. Mature pod yellow. Seed coat glossy, translucent, umbilical black. Creamy white, medium to thick. A hundred grains weigh about 200 g. Crude protein content was 31.19%, starch 37.2%, fat 0.96%, crude fiber (dry base %) 8.1%. Spring, middle and late ripening varieties. During the production test from 2007–2008, the average yield was 4445.25 kg/ha.	Suitable for planting in irrigated land with elevation of 2300–2600 m in Qinghai, Gansu, Tibet provinces in China.
Linxi 1	The plant height was 100.0 ~ 120.0 cm. Primary leaves ovoid, green, stipules pale green, compound leaves oblong, average number of leaflets 4–5. Main stem anthesis 6 ~ 7, terminal anthesis 10 ~ 11, flag white, pale brown veins, white wings, keel green. There were 8.0 ~ 9.0 effective pods per plant, and the pod was in the semi-erect pod state. The pod was of large pod type, with fresh pod length of 15.2 ~ 17.5 cm, pod width of 3.5 ~ 5.0 cm, mature pod length of 10.0 ~ 10.5 cm, and pod width of 2.5 ~ 3.0 cm. Each pod contains 1.0 ~ 3.0 granules. Mature pods are dark brown. Seed coat light green, dull, umbilical black, broad thin – shaped. Fresh grain length: 3.5 ~ 4.0 cm, width: 2.50 ~ 3.05 cm; seed length: 2.4 ~ 2.5 cm, width: 1.70 ~ 1.75 cm, 100-seed weight: 195.0 ~ 200.0 g, crude protein content: 28.85%, starch content: 46.24%, fat content: 1.227%, crude fiber content: 7.099%. It belongs to semi – winter, middle and early maturity, and its growth period is 96 ~ 104 d. From 1999–2000, the average dry seed yield was 2512.2 kg/ha after multi-point identification in Qinghai province. In 2000, the average fresh pod yield was 15132.7 kg/ha after participating in the production test and demonstration of faba bean in Qinghai province.	Faba bean autumn sowing area and spring sowing area can be planted, in China.
Qinghai 12	Plant height 104.4 ~ 145.3 cm. Primary leaves ovoid, green; stipules pale green. Compound leaves long elliptic, average number of leaflets 4 ~ 5. Flowers with white flag, pale brown veins, white wing, with a black disk in the center, green keel.14 ~ 15 effective pods per plant. Pod set in semi-erect form. Pod length 10.0 ~ 12.0 cm, pod width 2.0 ~ 2.4 cm. Each pod contains 2.1 ~ 2.3 grains. Mature pod black. Seed coat glossy, translucent, umbilical black. Creamy white, medium to thick. The seeds were 2.1 ~ 2.3 cm long, 1.7 ~ 2.0 cm wide, and 100 seeds weighed 195.0 ~ 200.0 g. The content of crude protein was 26.50%, starch 47.58%, fat 1.47% and crude fiber 7.37%. Spring, medium maturity variety, growth period 110 ~ 125 d. From 2001–2002, the average yield of 5070.0 kg/ha was increased by 8.18% compared with that of Qinghai 10. From 2003–2004, the average yield of 4218.0 kg/ha was increased by 6.7% compared with that of Qinghai 10.	It is suitable for planting in the irrigated agricultural area with an elevation of 2000–2600 m and the middle mountain dry land, in Qinghai province and northwest China.

(continued)

Cultivar	Important traits	Cultivation location
Qinghai 11	Plant height 140.0 ~ 145.0 cm. Primary leaves ovoid, green; stipules pale green. Compound leaves long elliptic, average number of leaflets 4 ~ 5. The main stem starts with 4 ~ 5 knots and ends with 11 ~ 12 knots. Flag white, pale brown veins, white pterygium, with a black central disk, keel greenish white. There are 20 ~ 25 effective pods per plant. Pod set in semi – erect form. Pod length 7.5 ~ 9.0 cm, pod width 2.5 ~ 2.7 cm. Each pod contains 1.8 ~ 2.0 capsules. Mature pod black. Seed coat glossy, translucent, umbilical black. Creamy white, medium to thick. The seeds were 2.13 ~ 2.22 cm in length, 1.88 ~ 2.00 cm in width and 190.0 ~ 195.0 g in weight. The content of crude protein was 25.66%, starch was 45.35%, fat was 1.38% and crude fiber was 6.20%. Spring, medium maturity variety, growth period 110 ~ 120 days. From 1999–2000, the average yield of 4333.5 kg/ha was increased by 12.3% compared with the control. From 2001–2002, the average yield of 5293.5 kg/ha was increased by 18.9% compared with the control.	It is suitable for planting in irrigated agricultural areas below 2600 m above sea level and on high water land, in Qinghai province and northwest China.
Lincan 7	It is belonging to medium big grain varieties, spring, growth period of 120 d or so, plant height 140 cm, branch 1–3, 1 cm thick stems, young stem color green, leaf blade elliptic, leaf color shallow green, shallow purple flowers, pod height 25 cm in the beginning, pod number per 10–18, each pod 2–3 grain, grain number per 20–40 grains, pod 11 cm long, 2.1 cm wide, pods grain of 2.3 cm long, 1.65 cm wide, hundred grain weight 186.9 g, grain full neat, kind of milky white skin, hilum black. The protein content was 29.04%, lysine 1.81%, starch 42.7%, tannin 0.59%. Resistant to root rot, like fertilizer and water. In 2007, the yield was 3810–5025 kg/ha in 5 stations, and the average yield was 4497 kg/ha, which was 12.36% higher than CK, and the increase was 9.06–14.3%.	Suitable for Gansu, Qinghai, Ningxia, Inner Mongolia, Xinjiang, Sha'anxi, Sichuan Aba spring faba bean production areas in China.
Lincan 10	Belong to medium big grain varieties, plant type is compact, plant growth and tidy, spring, growth period about 120 d, plant height 125 cm, branch 1–3, 1 cm thick stems, young stem color green, leaf blade elliptic, leaf color shallow green, shallow purple flowers, pod height 25 cm in the beginning, pod number per 10–18, each pod 2–3 grain, grain number per 20–40 grains, pod 11.5 cm long, 2.2 cm wide, pods grain of 2.3 cm long, 1.65 cm wide, hundred grain weight 182.56 g (try the province average) for 2 years, The seeds are plump and neat, the seed coat is milky white, and the seed navel is white. According to the test report of agricultural testing center of Aansu Academy of Agricultural Sciences, contains 10.93% water, 31.76% crude protein, 1.01% lysine, 54.661% starch, 0.863% crude fat and 0.601% tannin. From 2011–2012, the 2-year average yield was 2982 kg/ha, increasing 12.5% compared with Lincan 5 and 24.16% compared with Lincan 2.	Suitable for Gansu, Qinghai, Ningxia, Inner Mongolia, Xinjiang, Sha'anxi, Sichuan Aba spring faba bean production areas in China.

Maya Candou	Plant height: 130.0 ~ 140.0 cm. Primary leaves ovoid, green; stipules pale green. Compound leaves long elliptic, average number of leaflets 4 ~ 5. Flowers with white flag, pale brown veins, white wing, with a black disk in the center, green keel. The effective pod number per plant was 14 ~ 18, pod was hypo erect, pod length was 8.0 ~ 10.4 cm, pod width was 1.8 ~ 2.0 cm, pod size was 1.9 ~ 2.0, mature pod was black and brown. Seed coat milky white, shiny, translucent, umbilicus black, medium thickness, granule like horse teeth, seeds 2.3 ~ 2.5 cm long, 1.3 ~ 1.4 cm wide, 100 seeds weight 130.0 ~ 140.0 g. The content of crude protein, starch and crude fat was 28.2, 47.3 and 1.48%, respectively. It belongs to spring and medium mature varieties, with growth period of 110 ~ 120 days. From 2002–2003, he participated in the regional identification test of horse tooth faba bean, with an average yield of 4231.8 kg/ha, and an average yield increase of 6.1% compared with the control.	It is suitable for planting in the irrigated agricultural area with an elevation of 2500 ~ 2900 m and the middle mountain dry land, in western part of China.
Tong Can Xian 7	The whole growth period was about 220 d (the growth period of fresh pod was about 209 d). The seedling stage growth potential is prosperous, the plant high school and so on slants, resists the fertilizer to resist to fall, the straw green seed is ripe, does not crack the pod, is ripe good. In general, the height of the adult plant was about 96 cm, with many branches, 4.6 effective branches per plant, 15.2 pods per plant, among which 19.5% was one pod and 80.5% was more than two pods. The fresh pod was 11.81 cm long and 2.55 cm wide. Fresh seeds were 3.01 cm long and 2.18 cm wide. In the trial area, the fresh weight of 100 pods was 2500 g, the fresh seed weight was 379.3 g, the design and color of light purple flowers (partial white flowers), the dry seed coat was white (slightly greenish white excessive color at the time of harvest), the black navel, the seeds were large, and the dry seed weight was about 205 g. High quality, high protein and starch content, protein (dry base) content of 29.7%, fat 1.2%, starch (dry base) content of 53.8%. In the 2-year regional experiment, the average yield of fresh pod was 17.78 mt/ha, which was 7.44% higher than that of CK (a local variety). Fresh seeds/pods weight averaged 33.94%. Fresh seed yield was 6.04 mt/ha, 9.32% higher than that of CK. From 2010–2011, the high-yielding cultivation experiment and demonstration of this variety showed that the average fresh pod yield was 18.73 mt/ha, and the dry seed yield was 3.07 mt/ha.	Wide adaptability, can be in Jiangsu, Zhejiang, Shanghai, Fujian, Anhui, Hubei, Chongqing, Sichuan and other autumn sowing faba bean ecological areas, especially suitable for suburban food faba bean cultivation.
Tongcanxian 6	Winter, medium maturity varieties, the whole growth period of 220 d, coastal areas of fresh pods on the market in late April to mid-May, early 2 ~ 3 d than Japan CK (Japan Cun Can). Seedling flourishing, plant height of 85 cm, purple flowers. There were 3.9 effective branches per plant, 9 pods per plant, one pod accounted for 33.6%, and more than two pods accounted for 66.4%. The pods were 10.4 cm long and 2.8 cm wide, with an average weight of 2241.5 g. The fresh seeds were 3.0 cm long and 2.2 cm wide, and the 100-grain weight of the fresh seeds was 429.6 g. Dry seeds weigh about 200 g and contain 30.2% crude protein. Black navel, seed coat light purple, can be used for purity identification. In 2005, the fresh pod yield was 15.72 mt/ha, ranking the first among the tested varieties, and the yield was 36.8% higher than that of Japan CK. The yield of fresh seeds was 4.95 mt/ha, which was 30.95% higher than that of CK, ranking first. Fresh seeds/pods weight averaged 31.5%.	Wide adaptability, can be in Jiangsu, Zhejiang, Shanghai, Fujian, Anhui, Hubei, Chongqing, Sichuan and other autumn sowing faba bean ecological areas, especially suitable for suburban food broad bean cultivation.

(continued)

Cultivar	Important traits	Cultivation location
Tong Can Xian 8	The whole growth period of broad bean was about 220 d (the growth period of fresh pod was about 208 d). The seedling stage growth potential is prosperous, the plant high school and so on slants, resists the fertilizer to resist to fall, the straw green seed is ripe, does not crack the pod, is ripe good. In general, the height of the adult plant was about 94 cm, with many branches, 5.15 effective branches per plant, 14.7 pods per plant, of which one pod accounted for 23.5%, and more than two pods accounted for 76.5%. The fresh pod was 11.26 cm long and 2.49 cm wide, and the fresh seed was 2.83 cm long and 2.06 cm wide. The average 100 fresh pods weight was 2346 g, and the average fresh seed weight was 379.5 g. Light purple flowers (white flowers), black umbilical, and white seed coat of dry seeds were larger, and the dry seed weight was about 195 g. Good quality, high protein and starch content, protein content of 27.9%, fat 1.2%. The average yield of fresh pod was 17.42 mt/ha, which was 5.31% higher than that of Japan CK. Fresh seeds/pods weight averaged 33.26%. Fresh seed yield was 5.83 mt/ha, 5.43% higher than the control. From 2010–2011, the high-yield cultivation experiment and demonstration of this variety showed that the average fresh pod yield was 18.08 mt/ha, and the dry seed yield was 2.98 mt/ha.	Wide adaptability, can be in Jiangsu, Zhejiang, Shanghai, Fujian, Anhui, Hubei, Chongqing, Sichuan and other autumn sowing broad bean ecological areas, especially suitable for suburban food broad bean cultivation.
Haimen Daqingpi	Winter, medium maturity varieties, the whole growth period of 221 d. Plant shape compact, erect growth, stout stem, plant high school, general plant height of 90 cm, purple flowers. There were many branches, 4.5 branches per plant, 12.2 pods per plant, 1.6 seeds per pod, and the pod length was 8.0 cm. The seeds are large, flat, broad and thin, 2.03 cm long and 1.52 cm wide, green and shiny seed coat, black umbilicus, slightly uplifted at the base, generally weighing about 115 ~ 120 g. Dry seed protein content of 25% ~ 30%, crude fat 1.68% ~ 1.98%, cold – resistant, disease – resistant, ripe good. Can be mono cropped, but also with corn, cotton, vegetables, medicinal materials, such as inter-planting. The yield of this variety is 2700 kg/ha in mono cropping and 2250 kg/ha in inter-planting with cotton and corn. High yield cultivated plot, mono cropping yield 3300 kg/ha, inter-planting 2700 kg/ha.	Broad adaptability, can be in the Jiangsu broad bean ecological area and the Yangtze river in the middle and lower reaches of broad bean ecological area, can also be in suburban counties for high-quality fresh broad bean cultivation.
Jian Li Xiao Can Dou	Winter, late maturity varieties, the whole growth period of 212 d. Prostrate growth type, plant height 157.7 cm, color white/purple; The number of stems and branches per plant was 4.2, the number of pods per plant was 30.5, the length of pod was 7.77 cm, and the number of single pod was 2.36. Seed coat dark green, green, navel black, brown, granular thick; 100 dry seeds weigh 61.73 g. Good yield and stability. Average dry grain yield 41.10 g per plant; The cultivated yield in the plot is 2195 ~ 2996 kg/ha.	Wide adaptability, in the Yangtze river basin autumn sowing areas can be planted production.

Yundou 825	It is an autumn sowing medium – ripe large – grain variety. 188–202 d during the whole growth period, with unlimited flowering habits, the seedlings branched upright and plant height of 101.5 cm; Plant type compact, young stem green, mature stem brown yellow, medium branching force, average branching number 2.95 branches/plant; Small foliage shape oval, round leaves yellow green color, design and color is white, the pod hard, pod shape oblate bucket, fresh green pods and mature pods for shallow brown, skin white, hilum white, cotyledon yellow-white, grain shape wide thick, 9.9 pods per plant and single pod 1.30 grain, the 100 grain weight of 144.9 g and 20.2 g grain weight per plant, dry grain starch content 49.74%, crude protein content 24.12%, and 61.2% of the total sugar content, tannin content 0.025%; Because the total sugar content is high, the tannin content is low, the processing quality is extremely excellent. The average dry grain yield of 3892.1 kg/ha was 10.76% higher than that of CK. Field production experiment of dry grain yield 3430–3591 kg/ha; The average yield was 4670 kg/ha, with an increase rate of 1.1–15.3%.	Faba bean producing areas and similar habitats in Yunnan province at an elevation of 1100–2300 m.
Fengdou 13	It is an autumn sowing medium – ripe large – grain variety. Whole growth period 185 d, unlimited flowering habit; Seedlings branched upright, plant height of 78.25–99.42 cm; young stem purplish red, mature stem brown yellow. The average branch number was 4.1 branches/plant. Small foliage long elliptic, leaf light green color, design and color is purple, pod quality thin, tender pod shape oblate bucket, pod 8.67 cm long, bright green pods and mature pods for shallow brown, skin white, hilum white, cotyledon yellow-white, grain shape wide thick, 10.0 pods per plant and single pod 1.7 grain, the 100-grain weight is 138.5 g and 21.3 g grain weight per plant, dry grain starch content is 30.4%, crude protein content is 40.6%. Belong to high protein content variety. The average dry grain yield of the regional test in Yunnan province was 4419.45 kg/ha, 4.78% higher than that of CK. Field production experiment on average 4119.3 kg/ha, dry grain yield increase rate was 20.76%.	Yunnan province, 1600–2200 m above sea level. And similar habitat area cultivation.
Fengdou 10	It is an autumn sowing medium – ripe large – grain variety. Whole growth period 180 d, unlimited flowering habit; Seedling branches erect, plant height 96.5–139.6 cm; Young stem green, mature stem brown yellow; The average branch number was 4.1 branches/plant. Small foliage shape long oval, round leaf color is green, design and color is white, the pod hard, pod shape oblate bucket, pod 9.5 cm long, bright green pods and mature pods for shallow brown, skin white, hilum white, cotyledon yellow-white, grain shape wide thick, 9.5 pods per plant, single pod 1.81 grain, the 100-grain weight 133.2 g, 18.1 g grain weight per plant, dry grain starch content 49.68%, crude protein content is 28.19%, belong to high protein content variety. The average dry grain yield of the regional test in Yunnan province was 4080 kg/ha, which was 7.1% higher than that of the control variety 8010. Dry grain yield an average of 4620 kg/ha in field production experiment, increase production rate of 7.6–19.2%.	Broad bean producing areas in Yunnan province with an elevation of 1300–2100 m; And similar habitat area cultivation.

(continued)

Cultivar	Important traits	Cultivation location
Yundou 324	It is an autumn sowing medium – ripe large – grain variety. The whole growth period was 193 d, the flowering habit was unlimited, the branch of seedlings was semi-erect, the branch force was strong, and the average branch number was 3.7 branches/plant. Plant height 80–100 cm, compact plant, young stem lilac red, mature stem brownish yellow, small leaves ovoid, leaves yellow-green, flowers lilac, pod hard, pod oblate barrel; Fresh pod green, mature pod light brown; Seed coat green, seed hilum green; Granule width and thickness, cotyledons yellow and white; Single plant 9.92 pod, single pod 2.39 grain, 100-grain weight 132 g, single plant grain weight 31.4 g, dry grain starch content 45.88%, crude protein content 25.59%, tannin content 0.06%, fresh grain soluble sugar content 13.6%. It is a kind of vegetable with high quality. Strong frost resistance and moderate drought resistance. Field production experiment dry grain yield 3723–4596 kg/ha, the average yield of 4159 kg/ha, increase production rate of 7.5 42.1%, fresh pod yield 20,535–33,000 kg/ha.	Suitable for Yunnan, Sichuan and Guizhou areas with an altitude of 1100–2400 m for autumn sowing, and 1800–3100 m for spring sowing and summer sowing. It can be cultivated in autumn sowing in Jiangsu and Zhejiang, central China and spring sowing in Gansu and Qinghai, 20–30 days earlier than local varieties.
Chengjiang Dabeidou	It is an autumn sowing medium – ripe large – grain variety. Whole growth period 186 d, unlimited flowering habit; Seedling branches semi creeping, plant height 107.5 cm; young stem green, mature stem brown yellow; The average branch number was 2.96. Plant type compact, small leaf shape long oval, green leaf color, white flower color, hard pod, pod shape oblate barrel, fresh pod greenish-yellow, mature pod light brown, seed coat white, umbilicus white, black, cotyledon yellow-white, grain width and thickness, single plant 11.1 pod, single pod 1.60 seeds, 100-seed weight 131 g, single plant grain weight 20.6 g. Field production dry grain yield 3372–4635 kg/ha, the average yield of 4003.5 kg/ha, compared with the local cultivation of other similar local varieties, the average increase rate of 5.3%	Broad bean producing areas in Yunnan province with an altitude of 1300–1900 m; And similar habitat conditions of regional cultivation.

Yundou Zao 7	It is an autumn sowing early maturing large-grain variety. The whole growth period was 152–188 d, the flowering habit was unlimited, the seedlings branched upright, the plant height was 80.0 cm. Green stems, mature stem brown yellow, the branch power is strong, the average branch number 4.85 branch/plant, pod is hard, pod shape oblate bucket, fresh green pods and mature pods beige, seed coat is white, hilum white, cotyledon yellow-white, grain shape wide thick, 10.2 pods per plant, single pod 1.49 grain, the 100-grain weight is 130.6 g, per plant grain weight 16.2 g, dry grain starch content is 41.67%, the crude protein content is 26.8%. The dry grain yield is 4200–6600 kg/ha in the field production experiment; The average yield of 4400 kg/ha, increase production rate of 30–72.9% to the CK, the highest fresh pod yield is 23,100 kg/ha.	In Yunnan province, in the normal season is below the elevation of 1600 m, or the cultivation in the production area of off-season faba bean at the elevation of 1100–2400 m, and the cultivation in the region similar to the habitat.
Yundou 147	It is an autumn sowing medium – ripe large – grain variety. Whole growth period 190 d, unlimited flowering habit, seedling branching creeping, plant height 79.08 cm, plant type compact; The average branch number was 3.64. The young stem was green, the mature stem was red-green, and the leaf color was dark green. Leaflets are long and round, white in design and color, hard in pod, flat in pod shape, greenish-yellow in fresh pod, light brown in mature pod, white in seed coat, black in umbilicus, yellow-white in cotyledon, broad and thick in grain shape, 11.4 pods per plant, 1.93 seeds per pod, 127.38 g in 100 seeds, 23.68 g in single plant, starch content 47.69% in dry seeds, 26.21% in crude protein. Strong frost resistance, moderate drought resistance. The average yield of the regional experiment in Yunnan province was 3475.5 kg/ha, which was 21.7% higher than that of the control species 8010. 3028–4812.5 kg/ha in field production experiment, the average yield of 3920 kg/ha, increase production rate of 11.2–41.5%.	Suitable for Yunnan province elevation 1100–2400 m autumn sowing, 1800–3100 m summer sowing faba bean producing areas, and similar habitat area planting.
Chenghu 19	Medium and early maturing varieties, growth period 183 d. Unlimited pod bearing habit, vigorous growth, upright growth. Young stem light purple, mature stem green, plant height 114.9 cm. Main stem branched 2.4, leaf color is dark green, elliptic, flower purple. Single plant bearing 25.4 pods, 7.0 cm long, 2.6 cm wide, hard pod, slightly curved, mature pod black, single pod number of 2 or more. The newly harvested dry seeds were narrow and thick in shape, with pale green seed coat, black umbilicus, and 100-seed weight of 112.5 g. The dry grain protein content was 32.5% and the fat content was 1.25%. During the regional test in Sichuan province from 2007–2008, the average yield was 1859 kg/ha, which was 12.1% higher than that of the control Chenghu 10. In the production test in Sichuan province in 2008, the average yield was 2153 kg/ha, which was 15.3% higher than that of the control Chenghu 10, and the yield at Neijiang point reached 2196 kg/ha.	It is suitable for planting in different farming systems in Sichuan province.

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Cultivar	Important traits	Cultivation location
Chengdu Dabai	Early maturity variety, growth period 170 d. Habit of bearing unlimited pods and growing upright. Young stem light purple, mature stem green. The plant was 103 cm tall, with 4 main stem branches, dark green leaves, oval leaves and purple flowers. Each plant had 20.8 pods, with a pod length of 6.5 cm and a width of 3.1 cm, hard pod, straight pod and black mature pod. The newly harvested dry seeds were broad and thick, with milky seed coat, mostly white navel and a few black navel, and the weight of 100 seeds was 106.9 g. Protein content in dry grains was 28.4%. General output 1500 kg/ha, minimum 1275 kg/ha, maximum 1875 kg/ha.	Slopes of hilly area, with wet sand soil, yellow soil, in winter growing areas.
Zhijin Xiaoqingpi	Winter, middle and late ripening varieties, whole growth period 209 day. Semi creeping growth type, plant height 60.7 cm, flowers purplish red; The number of stem branches per plant was 2.40, the number of pod bearing per plant was 9.70, the pod length was 6.63 cm, and the number of single pod was 2.23. Seed coat light green, seed navel black, granular thick; Dry seeds weigh 70.93 g. Good yield and stability. Average dry grain yield per plant 22.34 g; The cultivated yield in the plot is 2300 ~ 2760 kg/ha.	Wide adaptability, in the Yangtze river basin autumn sowing areas can be planted for better production.

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Chapter 8
Hyacinth Bean (*Lablab purpureus* L. Sweet): Genetics, Breeding and Genomics

Panichayil V. Vaijayanthi, Chandrakant, and Sampangi Ramesh

Abstract Hyacinth bean (*Lablab purpureus* (L.) Sweet) is widely distributed in the Indian subcontinent, Africa and Southeast Asia. It is a multipurpose tropical legume valued as a vegetable, pulse, fodder and green manure crop. Despite a wide range of adaptability and diversity, it remains an underutilized crop. Broadening the genetic base and enhancing crop cultivar diversity is the key to sustainable production of hyacinth bean. Development of purelines through pedigree breeding is the preferred method of breeding in the hyacinth bean, as in other grain legume crops. Screening of germplasm resources, identification of trait-specific material and their use in breeding could be a long-term strategy to addressing various existing and anticipated production constraints. With the advent of molecular marker/omic technology, the pace and efficiency of hyacinth bean breeding has attained considerable momentum. DNA marker-assisted diversity analysis, chromosomal localization and unraveling of the mode of action of genes controlling traits of economic importance, tagging genomic regions controlling economic traits etc., will complement phenotype-based selection and breeding. Furthermore, deployment of various genomic tools will help in introgression of superior alleles into elite agronomic backgrounds and hence sustainable production of hyacinth bean.

Keywords Core set · Diversity · Field bean · Germplasm · *Lablab* · Legume

8.1 Introduction

Hyacinth bean (*Lablab purpureus* L. Sweet) is one of the oldest grain legumes grown in Asia, Africa and Australia (Ayyangar and Nambiar 1935). It is a bushy semi-erect perennial herb belonging to the family Fabaceae, subfamily Faboideae, tribe Phaseoleae and subtribe Phaseolineae. It is predominantly a self-pollinated crop with 2n = 22 chromosomes (Goldblatt 1981; She and Xiang 2015). *Lablab* is a monotypic genuswith a diverse history of origin and domestication. It is believed to

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have originated in India (Kukade and Tidke 2014; Nene 2006) or Africa (Maass 2016) and later introduced into China, West Asia and Egypt (Ayyangar and Nambiar 1935). It is also commonly known as field bean, dolichos bean, lablab bean, Indian bean, sem, bonavista bean, lubia bean, butter bean and Egyptian kidney bean in different parts of the world.

Hyacinth bean is highly popular in South Asia, Southeast Asia and Africa, where it is grown in rainfed agroecosystems (Haque et al. 2003; Rahman et al. 2002) as a vegetable, pulse, forage, cover and green manure crop (Adebisi and Bosch 2004). In China, it is very popular and has been grown on fences and trellises in backyards for centuries. In Bangladesh, it is the third most important vegetable in the central and southwestern parts of the country with a total cultivation area of 48,000 ha (Rashid et al. 2007). In India, hyacinth bean is primarily grown as a vegetable cum pulse rainfed crop in southern states such as Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra (Mahadevu and Byregowda 2005; Shivashankar and Kulkarni 1989). Immature grains (as a vegetable), dry grains (as a pulse in foods and snacks) (Ayyangar and Nambiar 1935; Shivashankar and Kulkarni 1989; Viswanath et al. 1971) and whole plant before flowering (as fodder for mulch and draught animals) (Magoon et al. 1974), are the economic products from hyacinth bean. It is a good source of dietary protein to vegetarians in South India. Hyacinth bean is an important food source in tropical Africa as well. In Kenya, the bean is popular as *njahe* and has historically been the main dish for breastfeeding mothers. It is popular as an ornamental plant in the USA and as forage in Australia.

8.2 Origin and Distribution

The center of origin of hyacinth bean has long been the subject of debate. According to several researchers, it is a native of Indian subcontinent (Kukade and Tidke 2014; Nene 2006) as documented in archaeobotanical studies from Hallur (2000–1700 B.C.) and Veerapuram sites (1200–300 B.C.), India (Fuller 2003). It is believed to have been introduced into China, West Asia and Egypt from India (Ayyangar and Nambiar 1935). However, Maass et al. (2005) suggested eastern and or southern Africa as the center of origin. In addition, they reported the intermediate nature of Indian wild collections, suggesting a pattern of domestication and distribution of hyacinth bean from Africa to Asia. Maass and Usongo (2007) further affirmed this hypothesis by studying seed characteristics of wild and cultivated forms. A dual center of origin hypothesis (Africa and India) is also postulated for hyacinth bean. However, the African continent shows greater occurrence of natural diversity of wild and cultivated forms. During later periods, the crop was domesticated and distributed to many countries like China, Indonesia, Malaysia, Egypt, Philippines, Sudan, Papua New Guinea, East and West Africa, the Caribbean, Central and South America (Fig. 8.1). Hoshikawa (1981) documented the introduction of hyacinth bean to Japan from China in 1654 where it is called *fujimame* and the young pods are used as a vegetable.

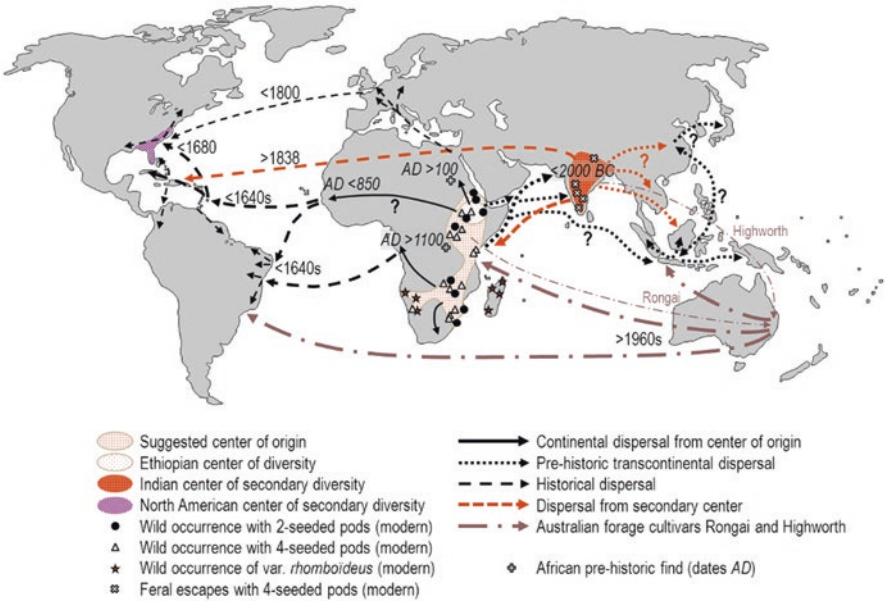

Fig. 8.1 Origin and distribution of hyacinth bean. (Source: Maass 2016)

8.3 Ecology

The hyacinth bean is remarkably adaptable to wide areas under diverse climatic conditions, such as arid, semiarid, subtropical and humid regions where temperatures are 22–35 °C, lowlands and uplands and many types of soils ranging from deep sands to heavy clays, and from acid to alkaline with a pH range of 4.4–7.8. Hyacinth bean prefers lower elevations but it can thrive up to 2100 m elevation. In the wild, hyacinth bean occurs in grassland, bushland and gallery forest, up to 2400 m elevation. It is a drought-tolerant crop, which grows well with the rainfall of 600–800 mm per annum. It has a deep tap root that can reach up to 2 m below the soil surface, permitting luxuriant growth in the dry season. It is normally a short-day plant, but day-neutral and long-day cultivars exist. Being a legume, it can fix atmospheric nitrogen to the level of 170 kg/ha (Ramesh and Byregowda 2016).

8.4 Taxonomy

Linnaeus used an ancient Greek adjective *dolichos*, meaning *long*, to describe a group of about 60 species of herbaceous plants and shrubs (Aleksandar and Vesna 2016). The first scientific name of hyacinth bean was *Dolichos lablab* L. and it is still being used as a synonym of *Lablab purpureus*. Adanson and Mochel (1763) for

Fig. 8.2 Two *Dolichos lablab* varieties: (**a**) *D. lablab* var. *typicus* Prain (=*Lablab purpureus* (L.) Sweet, (**b**) *D. lablab* var. *lignosus* Prain (=*Lablab purpureus* (L.) Sweet

the first time assigned the name *Lablab* for *Dolichos* L. *Lablab* is an Arabic name describing the dull rattle of the seeds inside the dry-pod. Roxburgh (1832) described the genus *Dolichos*, listing 7 varieties, of which 5 were cultivated and 2 were wild. *Dolichos lablab* var. *typicus* Prain (=*Lablab purpureus* (L.) Sweet and *Dolichos lablab* var. *lignosus* Prain (=*Lablab purpureus* (L.) Sweet were two subdivisions of cultivated varieties of hyacinth bean recognized by Purseglove (1968).

Dolichos lablab **var.** *typicus* **Prain [=***Lablab purpureus* **(L.) Sweet]** This crop is commonly known as Indian butter bean (Fig. 8.2a). It is a perennial twining herb widely distributed throughout the tropical and temperate regions of Asia, Africa and America. Pods are flat, long and tapering with long axis of seeds parallel to the suture. Mainly grown as a garden crop, and trained on a pendal for green soft whole pods (used as vegetable). It produces white, green or purple-margined pods with varying seed color (white, yellow, brownish, purple, black seeds).

Dolichos lablab **var.** *lignosus* **Prain [=***Lablab purpureus* **(L.) Sweet]** This crop is commonly known as Australian pea or field bean (Fig. 8.2b). It is a semi-erect, perennial herb, showing little or no tendency to climb. It bears pinnately trifoliate leaves, which are smaller than those of var. *typicus*. Inflorescence is a terminal raceme and flowers open in succession. Pods oblong, flat and broad, firm-walled and fibrous contain 4–6 seeds, with their long axis at right angles to the suture. Seeds almost rounded white, brown or black. The plant emits a characteristic odor.

Rivals (1953) proposed another classification of the cultivated species as (a) short-day varieties (photoperiod of 10–11 h) and (b) others (relatively unaffected by day length). Verdcourt (1970) recognized 3 subspecies: *unicinatus*, *purpureus*, *bengalensis*. Subspecies *unicinatus* produces small pods (40 × 15 mm) and is distributed in East Africa representing an ancestral form. However, the cultivated form belongs to ssp. *purpureus* and produces large pods (100 × 400 mm); ssp. *bengalen-*

sis has linear oblong-shaped pods (140 × 10–25 mm) and was domesticated in Asia. Although there were significant differences with respect to pod shape, it is presumed that ssp. *purpureus* and ssp. *bengalensis* are genetically very similar and most of the domesticated material in India belongs either to ssp. *purpureus* or ssp. *bengalensis*. Subspecies *uncinatus* was domesticated only in Ethiopia (Magness et al. 1971). Verdcourt (1980) revised the monotypic genus *Lablab* by combining the subspecies under in *Lablab purpureus* (L.) Sweet.

8.5 Botany

Growth Habit It is a perennial herb twining up to 1.5–8.8 m. However, bushy, semi-erect, and prostrate forms exist. Wide variation in form and habit compared to other legumes (Figs. 8.3 and 8.4) (www.lablab.org).

Roots Well-developed tap root system with many lateral and adventitious roots.

Stem Cylindrical, twining, hairy or glabrous, usually 2–10 m.

Leaves Alternate, trifoliate, leaflets ovate, often hairy. Very broad leaflets, ovate, leaf tip acuminate, slender and laterally compressed petioles.

Inflorescence Axillary raceme with many flowers. Peduncle glabrescent, 1–5 flowers together form tubercles on rachis, deciduous, ovate to elliptic, short pedicels with 2 bracteoles attached at the base of the calyx.

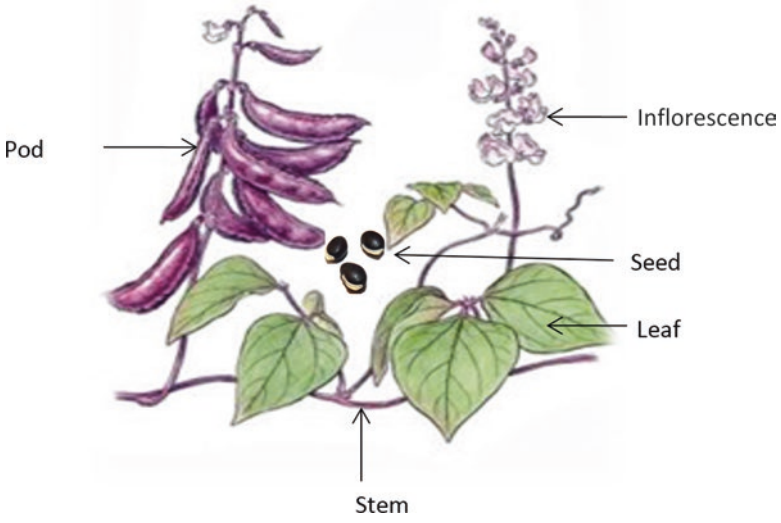

Fig. 8.3 Schematic representation of hyacinth bean plant

Fig. 8.4 Variability for growth habit among hyacinth bean germplasm collection

Flowers White, pink, red or purple colored, in clusters of 4–5, each with 2 large basal bracts, stamen free, long, flattening and geniculate near the base. Anthers are uniform, ellipsoid diadelphous (9 + 1), minutely denticulate and yellow. Sessile, finely pubescent ovary with 4 brown speckled ovules. Style abruptly upturned, laterally compressed, apical part thinly pubescent, persistent on pod, stigma capitate and glandular (www.lablab.org).

Pods Flat or inflated, pubescent or smooth, papery, straight, curved or crescent-shaped, white, green or purplish in color and approximately 5–20 cm long (Fig. 8.5) (www.lablab.org). Cultivars grown as a vegetable have thick fleshy pods with less fiber. Pods may be septate (each seed occupies a separate compartment in the pod) or nonseptate (pods have a bloated appearance).

Seed Each pod normally encloses 3–6 round, oval or flattened seeds. Seeds are variable in size and color (Fig. 8.6) ranging from white, red, brown, black or speckled hilum white, prominent and oblong, usually covering 1/3 of the seed. Germination is epigeal (www.lablab.org).

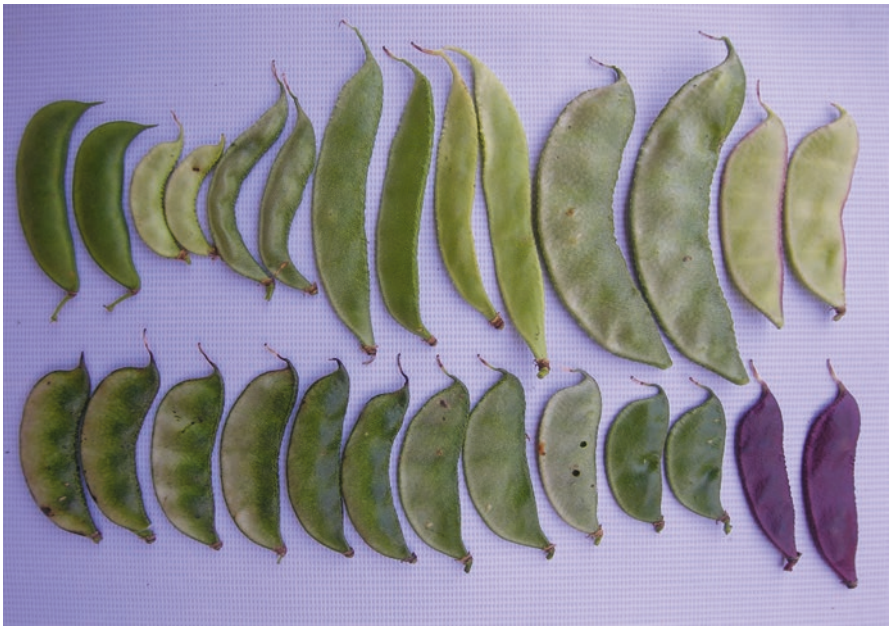

Fig. 8.5 Variability for pod size, shape and color among hyacinth bean germplasm collection

Fig. 8.6 Variability for seed size, shape and color among hyacinth bean germplasm collection

8.6 Cytology

Cytogenetic studies of hyacinth bean were primarily based on conventional staining techniques. Root tips from germinated seeds were collected, pretreated and fixed in acetic alcohol for slide preparation. Karyotype studies showed somatic chromosome number of 2n = 22 for hyacinth bean (Ali et al. 2011; Chen 2003). The chromosomal length varied from 1.17–3.00 μ (Ali et al. 2011). The haploid complement consisted of 11 metacentric chromosomes with 5 individually identifiable ones. Recently, FISH mapping of 5S and 45S rDNA in *Lablab purpureus* was reported (Iwata et al. 2013). She and Xiang, (2015) demonstrated genomic organization of hyacinth bean using sequential CPD staining and FISH with 5S and 45S rDNA probes. They depicted karyotype of hyacinth bean as $2n = 2x = 22 = 14$ m (2SAT) + 6sm + 2st (2SAT). These studies also revealed the presence of centromeric AT-rich heterochromatin and proximal GC-rich heterochromatin in hyacinth bean chromosomes. However, a molecular cytogenetic karyotype of this species is still unavailable.

8.7 Germplasm Collection, Conservation and Utilization

8.7.1 Germplasm Collection and Conservation

Diversifying the genetic base of crop cultivars is a prerequisite for continued genetic improvement to enhance productivity and to address various production constraints. More than 3000 hyacinth bean accessions have been collected worldwide (Maass et al. 2010); these genetic resources are preserved in the form of seeds in ex situ gene banks globally.

Table 8.1 Summary of hyacinth bean germplasm maintained in different countries, regions and institutes of the world

Countries/region/institute	Number of accessions	Source
South America	134	BI (2008)
North America, United States Department of Agriculture (USDA)	52	GRIN (2009)
Europe	82	BI(2008) and VIR (2009)
Oceania including Australia at Common Wealth Scientific and Industrial Research Organization (CSIRO)	104	BI (2008)
China	410	BI (2008)
Philippines	209	Engle and Altoveros (2000)
Taiwan at Asian Vegetable Research and Development Center (AVRDC)	423	AVRDC (2009)
Southeast Asia (countries other than Bangladesh and India)	82	BI (2008) and NIAS (2009)
Bangladesh	551	Islam (2008)
India at National Bureau of Plant Genetic Resources (NBPGR), New Delhi	221	BI (2008)
South Asia	93	BI (2008)
Ethiopia including International Livestock Research Institute (ILRI)	223	BI (2008)
Kenya	403	BI (2008)
Sub-Saharan Africa including International Institute of Tropical Agriculture (IITA), Nigeria	67	BI (2008)
University of Agricultural Sciences (UAS), Bengaluru, India	650	Byregowda et al. (2015) and Vaijayanthi et al. (2015a)

The National Gene bank of Kenya, Commonwealth Scientific and Industrial Research Organization (CSIRO-Australia), International Livestock Research Institute (ILRI-Ethiopia), International Institute of Tropical Agriculture (IITA-Nigeria), National Bureau of Plant Genetic Resources (NBPGR-New Delhi) and the University of Agricultural Sciences, Bengaluru (UAS (B)-India) hold the largest working germplasm collections of hyacinth bean. In Australia and New Zealand, only fodder types are maintained. Systematic efforts to collect, evaluate, catalogue, document and conserve hyacinth bean genetic resources in several countries/regions/institutes are summarized in Table 8.1 (Ramesh and Byregowda 2016).

8.7.2 Germplasm Utilization

From the above discussion, it is evident that the UAS, Bengaluru, India holds the largest working germplasm (650 accessions) of hyacinth bean. These accessions were characterized and evaluated for a set of 70 descriptors (16 vegetative, 14

Table 8.2 Status of hyacinth bean germplasm conserved at UAS, Bangalore, India

Sl. no	Geographical origin	Base collection[a]
1	Indian collections	544 (83.95%)
	(a) Karnataka	449
	(b) Andhra Pradesh	36
	(c) Maharashtra	14
	(d) Gujarat	36
	(e) Tamil Nadu	7
	(f) Kerala	2
2	Exotic collections	24 (3.7%)
3	Unknown Origin	80 (12.35%)
Total		648

[a]Figures within parenthesis indicate percentage of accessions

inflorescence, 20 pod, 20 seed traits) considering the spectrum of variability for these traits following the guidelines of Bioversity International (BI), (Byregowda et al. 2015). These descriptors can be used as diagnostic markers of germplasm accessions to maintain their identity and purity. They are also useful in conducting distinctness, uniformity and stability (DUS) testing, a mandatory requirement for protecting varieties under the Protection of Plant Varieties and Farmers' Rights (PPA & FR) Act of India, and similar laws in force in other countries (Byregowda et al. 2015).

Most traits of economic importance often exhibit high genotype × environment interactions and require multi-locational and multi-year evaluation, which is a resource-demanding task owing to the large size of the germplasm collections. Hence, Frankel (1984) proposed the concept of the *core collection*, a manageable representation of the base collection. A core collection is a subset of the entire collection chosen to represent the maximum genetic diversity with minimum redundancy (Brown 1989). Considering that the plant genetic resource collections being maintained at UAS, Bengaluru (Table 8.2) are large and pose difficulties in effective management and evaluation of accessions for quantitative traits, a core set (n = 64) which captures ≥90% of variability in the entire collection (n = 648) was developed (Vaijayanthi et al. 2015b) using Power Core (v.1), a program that applies advanced M-strategy with a heuristic search (Kim et al. 2007). The procedure adapted for the development of a representative core set is depicted in Fig. 8.7.

In similar efforts to reduce size and possible duplication, Bruce and Maass (2001) in Ethiopia and Islam et al. (2014) in Bangladesh, also developed core sets of 47–36 accessions from the base collections of 251–484 accessions, respectively. The core sets are considered a first look at sources of genetic resources for use in crop improvement programs. Because of their small size, core sets can be effectively characterized and evaluated across many locations/years and are considered ideal for discovering new sources of variation, identification of trait-specific accessions, gene discovery, allele mining and as an association mapping panel (Qiu et al. 2013; Upadhyaya 2015).

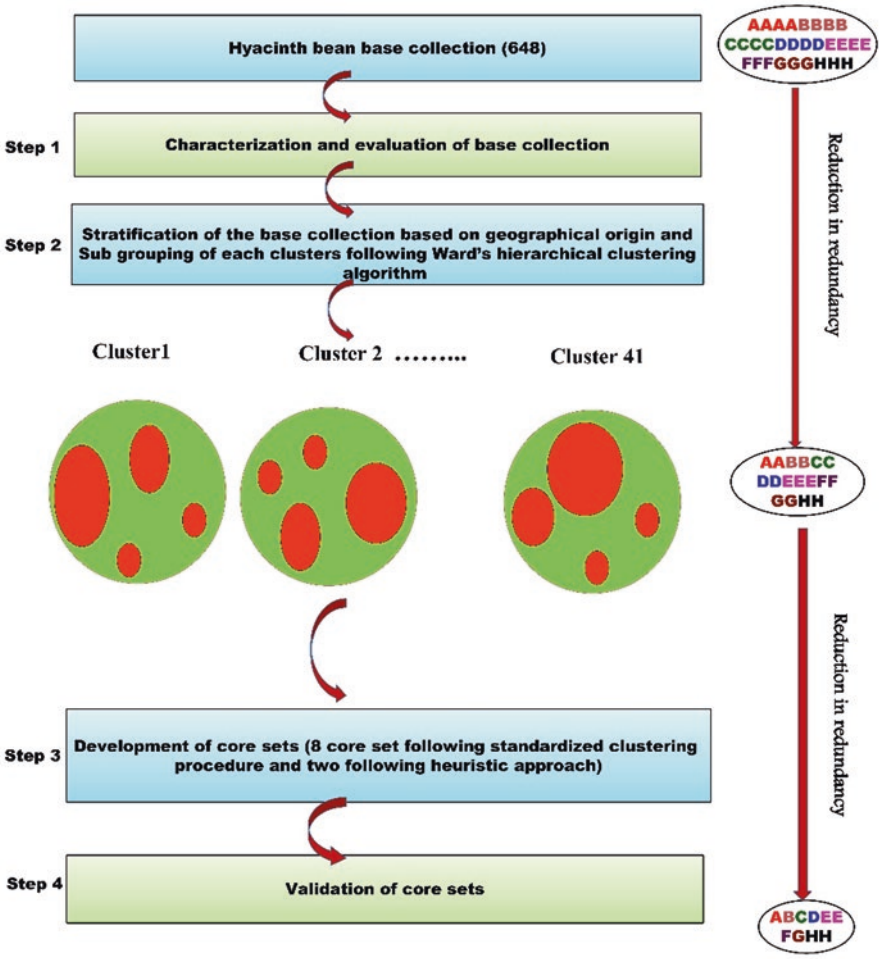

Fig. 8.7 Flow diagram depicting establishment of 10 core sets from 648 hyacinth bean accessions

In order to identify promising trait-specific germplasm accessions, the core set at UAS, Bengaluru was evaluated for 2 years (2012–2014) and those promising for per se productivity traits (Table 8.3) and multi-traits (Table 8.4) identified (Vaijayanthi et al. 2016a). Furthermore, promising germplasm accessions for multi-traits were evaluated in multi-locations to identify those widely/specifically adaptable to different agroclimatic zones. Accessions such as GL 250, FPB 35 and Kadalavare were found widely adaptable with a relatively high fresh pod yield (Vaijyayanthi et al. 2016b, 2017). These accessions are suggested for preferential use in breeding hyacinth bean varieties widely adaptable to different agroclimatic zones.

Table 8.3 Promising trait-specific germplasm accessions identified from core set

Traits	Germplasm accessions
Days to 50% flowering	HA-11-3, GL 326, HA-12-9, GL 432, GL 661
Primary branches plant^{-1}	GL 621, GL 199, GL 147, GL 228, GL 110, GL 12, GL 252, GL 205, GL 606, GL 527
Racemes plant^{-1}	GL 326, GL 142, GL 205, GL 447, GL 12, GL 530, GL 606, GL 199, GL 110, GL 438, GL 412
Fresh pods plant^{-1}	GL 447, FPB 35, GL 576, GL 418, KA, GL 142, GL 633, GL 527, GL 250, GL 66, GL 444
Fresh pod yield plant^{-1} (g)	GL 576, FPB 35, GL 527, GL 447, GL 142, GL 441, GL 66, GL 12, GL 418, GL 579
Fresh seed yield plant^{-1} (g)	FPB 35, GL 576, GL 527, GL 142, GL 447, GL 12
100 fresh seed weight (g)	GL 441, GL 6, GL 12, HA-12-9, GL 579, GL 142, GL 527, GL 68, GL 658, GL 66, GL 439, FPB 35

Table 8.4 Promising multiple traits specific germplasm accessions identified from core set

Identity of accessions	Traits
GL 576	Fresh pods plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1}
GL 110	Primary branches plant^{-1}, racemes plant^{-1}
GL 527	Primary branches plant^{-1}, fresh pods plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1}
GL 447	Racemes plant^{-1}, fresh pods plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1}
GL 142	Racemes plant^{-1}, fresh pods plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1}, 100 fresh seed weight
GL 441	Fresh pod yield plant^{-1}, 100 fresh seed weight
FPB 35	Fresh pods plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1}, 100 fresh seed weight.
GL 66	Fresh pods plant^{-1}, fresh pod yield plant^{-1},100 fresh seed weight
GL 12	Racemes plant^{-1}, fresh pod yield plant^{-1}, fresh seed yield plant^{-1}, 100 fresh seed weight.

8.8 Genetics of Important Productivity Traits

8.8.1 Qualitative Traits

Several researchers have reported the number and mode of action of genes controlling easily-observable/assayable growth traits, leaf traits, inflorescence traits, pod traits and seed traits in hyacinth bean (Table 8.5). Joint segregation analysis showed linkage among the genes controlling various qualitative traits. Independently segregating genes controlling direction of inter-nodal hairs, pod width, orientation of dry pods on the branches and seed color in hyacinth bean were found (Patil and Chavan

1961). On the contrary, genes controlling pod width and seed shape and those controlling orientation of dry pods and nature of pod surface are closely kinked without recovery of recombinants (Patil and Chavan 1961). This is possible because of the pleiotropic effect of a single gene. Raut and Patil (1985) reported a close linkage between genes controlling stem color and flower color, and between genes controlling flower color and leaf margin. The genes controlling photoperiod sensitivity and petiole color are linked with recombination of 33.16% and those controlling petiole color and growth habit and photoperiod sensitivity and growth habit are also linked with recombination of 32.92% and 6.14%, respectively (Rao 1987). The genes controlling growth habit and photoperiod sensitivity are linked with recombination of 7.82% (Rao 1987). On the other hand, genes controlling photoperiod sensitivity and stem color, growth habit and stem color segregated independently (Rao 1987). While the genes controlling photoperiod sensitivity and growth habit, photoperiod sensitivity and raceme emergence from the foliage and growth habit and raceme emergence from the foliage are linked in coupling phases with recombination of 29%, 24% and 21%, respectively; those controlling flower color and pod curvature are un-linked (Keerthi et al. 2016). The qualitative traits controlled by single/oligogenes could be used to identify true F_1s, to rule out the possibility of selfing due to the occurrence of pollination before opening of the flowers (Ayynagar and Nambiar 1935; Harland 1920; Kukade and Tidke 2014).

8.8.2 Quantitative Traits

Jacob (1981) reported partial dominance with a duplicate type of epistasis for green pod yield plant^{-1} and predominance of additive gene action for seed yield plant^{-1}. Rao (1981) reported the importance of all the three types of gene action (additive, dominant, epistatic) in different proportions in the inheritance of pod yield plant^{-1}, pods plant^{-1}, seed yield plant^{-1}, raceme length, pods raceme^{-1} and plant height. Muralidharan (1980) reported complementary epistasis with preponderance of dominance genetic variance (σ^2_D) in the inheritance of seed yield, while Reddy et al. (1992) documented the preponderance of additive genetic variance (σ^2_A) for number of pods plant^{-1}. Khondker and Newaz (1998) reported the predominant role of additive variance in the inheritance of days to flowering, pod width, seeds pod^{-1} and 20-pod weight. On the other hand, traits such as number of inflorescences plant^{-1}, number of pods inflorescence^{-1} and pod yield plant^{-1} were mostly governed by σ^2_D.

Sakina and Newaz (2003) reported the preponderance of σ^2_A in the inheritance of all the characters considered for the study and presence of complete dominance in controlling flowering time and partial dominance for raceme plant^{-1} and number of flowers raceme^{-1}. Alam and Newaz (2005) reported the importance of both σ^2_A and σ^2_D in the expression of flower and pod traits. Raihan and Newaz (2008) also documented the importance of both σ^2_A and σ^2_D with a preponderance of σ^2_A in the expression of all the traits except number of inflorescences plant^{-1}. Desai et al. (2013) reported preponderance of σ^2_A for all the traits considered for the study

Table 8.5 Summary of genetics of different traits in hyacinth bean

Traits and different states	Number of genes	F_2 ratio	Mode of action	References
Growth traits				
Orientation of stem inter-nodal hairs	1	3 Downward:1 upward	Downward > upward	Ayynagar and Nambiar (1935)
Upwards/downwards				
Orientation of inter-nodal hairs	1	3 Downward: 1 upward	Downward > upward	Patil and Chavan (1961)
Upwards/downwards				
Stem pigmentation on nodes and internodes	3	27 Pigmented: 37 non-pigmented	Pigmented > non-pigmented	Manjunath et al. (1973)
			Complementary epistasis	
Growth habit	1	3 Spreading: 1 compact	Spreading > compact	Rao (1987)
Spreading/compact				
Growth habit	3	57 Erect: 7 prostrate	Erect > prostrate	Girish and Byregowda (2009)
Erect/prostrate				
Growth habit	2 or 3	9 Indeterminate: 7 determinate (two complementary genes)	Indeterminate > determinate	Keerthi et al. (2014a) and Keerthi et al. (2016)
Determinate/indeterminate		57 Indeterminate: 7 determinate (1 basic and two complementary genes)	Complementary epistasis	
Stem color	1	3 Purple: 1 green	Purple > green	Raut and Patil (1985)
Purple/green				
Stem color	3	57 Purple: 7 green	Purple > green	Rao (1987)
Purple/green			Complementary epistasis	
Leaf traits				
Leaf margin color	1	3 Purple: 1 green	Purple > green	Raut and Patil (1985)
Purple/green				

Leaf vein color	2	9 Purple: 7 green	Purple > green	Raut and Patil (1985)
Purple/green			Complementary epistasis	
Petiole color	2	9 Purple: 7 green	Purple > green	Rao (1987)
Purple/green			Complementary epistasis	
Leaf color	3	54 Dark green: 10 green	Dark green > green	Girish and Byregowda (2009)
Dark green/green			Complementary epistasis	
Leaf texture	2	9 Rough:7 smooth	Rough > smooth	Girish and Byregowda (2009)
Rough/smooth			Complementary epistasis	
Inflorescence traits				
Photoperiod sensitivity to flowering	1	3 Sensitive: 1 insensitive	Sensitivity > insensitivity	Rao (1987)
Sensitive/insensitive				
Photoperiod sensitivity to flowering	1	3 Sensitive: 1 insensitive	Sensitivity > insensitivity	Prashanti (2005)
Sensitive/insensitive				
Photoperiod sensitivity to flowering	1	3 Sensitive: 1 insensitive	Sensitivity > insensitivity	Keerthi et al. (2014a) and Keerthi et al. (2016)
Sensitive/insensitive				
Flower color	1	3 Purple: 1 white	Purple > white	Raut and Patil 1985)
Purple/white				
Flower color	1	3 Purple: 1 white	Purple > white	Keerthi et al.(2016)
Purple/white				

(continued)

Table 8.5 (continued)

Traits and different states	Number of genes	F_2 ratio	Mode of action	References
Raceme emergence from foliage	2	13 Emerge out of the foliage: 3 remain within the foliage	Emergence > remaining within the foliage	Keerthi et al. (2016)
Emerge out of the foliage/ remain within the foliage			Inhibitory epistasis	
Pod traits				
Orientation of dry pods to branches	1	3 Erect: 1 dropping	Erect > drooping	Ayynagar and Nambiar (1935)
Erect/drooping				
Width of pods	1	3 Medium: 1 narrow	Medium width > narrow width	Ayynagar and Nambiar (1935)
Medium/narrow				
Pod width	1	3 Broad: 1narrow	Broad > narrow	Patil and Chavan (1961)
Broad/narrow				
Nature of surface of narrow pods	1	3 Septate: 1 nonseptate	Septate > non-septate	Ayynagar and Nambiar (1935)
Septate/non-septate				
Pod position after drying	2	15 Erect: 1 dropping	Erect > drooping	Patil and Chavan (1961)
Erect/drooping			Duplicate dominant	
Nature of pod surface	2	15 Smooth: 1 shriveled	Smooth > shriveled	Patil and Chavan (1961)
Smooth/shriveled			Duplicate dominant	
Pod color	1	3 Green: 1 light green	Green > light green	D' cruz and Ponnaiya (1968)
Green/light green				
Pod shape	1	3 Flat: 1 bloated	Flat > bloated	D' cruz and Ponnaiya (1968)
Flat/bloated				

Pod curvature	4	117 Straight: 139 curved	Curved > straight	Girish and Byregowda (2009)
Straight/curved			Two complementary, one inhibitory and one anti-inhibitory	
Pod curvature	2	9 Straight: 7 curved	Straight > curved complementary, epistasis	Keerthi et al. (2016)
Straight/curved				
Pod fragrance	2	13 High: 3 low	High > low	Girish and Byregowda (2009)
High/low			Inhibitory epistais	
Seed traits				
Seed coat color	1 or 2	9 Khaki: 3 chocolate: 1 buff	Khaki > chocolate > black > buff	Ayynagar and Nambiar (1936 a, b)
Khaki/chocolate/black/buff			Supplementary epistasis	
Seed shape	1	3 Fat: 1 round	3 Flat > 1Round	Patil and Chavan (1961)
Round/flat				
Seed color	1	3 Red: 1 white	Red > 1white	Patil and Chavan (1961)
Red/white				
Seed coat color	1	3 Chocolate: 1 brown	Chocolate > brown	D' Cruz and Ponnaiya (1968)
Chocolate/brown				

Source: Ramesh and Byregowda (2016)

except days to 50% flowering and number of pods cluster^{-1}. Das et al. (2014) reported the importance of σ^2_A in the inheritance of number of inflorescences plant^{-1} and number of nodes inflorescence^{-1}. On the contrary, length of inflorescence, number of pods inflorescence^{-1}, pod length and number of seeds pod^{-1} were influenced by σ^2_D, while the characters such as days to 50% flowering, number of pods plant^{-1}, pod weight and pod yield plant^{-1} were controlled by both σ^2_A and σ^2_D. Keerthi et al. (2015) reported the predominance of σ^2_A in the inheritance of racemes plant^{-1} and predominance of σ^2_D in the inheritance of pod weight plant^{-1}. Additive genetic variance was found to be very important in the inheritance of days to flowering and seed weight plant^{-1}. Furthermore, Keerthi et al. (2015) documented not only the important role of epistasis but also significant bias in the estimates of both σ^2_A and σ^2_D for most of the traits investigated. It is therefore not advisable to ignore epistasis in studies designed to estimate σ^2_A and σ^2_D controlling quantitative traits. Identification and non-inclusion of the genotypes that contribute significantly to epistasis could be a better strategy to obtain unbiased estimates σ^2_A and σ^2_D. Selection based on unbiased estimates σ^2_A and σ^2_D is expected to be reliable and effective. Alternatively, one or two cycles of bi-parental mating in the F_2 generation is expected to dissipate epistasis and selection will be effective (Chandrakant et al. 2015).

8.9 Breeding for Hyacinth Bean Improvement

Hyacinth bean has evolved as a highly-photoperiod-sensitive crop requiring long-nights (short-days) for switching over from a vegetative to a reproductive phase (Ayyangar and Nambiar 1935; Kim and Okubo 1995; Kim et al. 1992; Schaaffhausen 1963; Shivashankar and Kulkarni 1989; Viswanath et al. 1971). Most varieties grown by Indian and African farmers are landraces which are highly-photoperiod sensitive (PS) and display indeterminate growth habit. Indeterminacy is advantageous for subsistence production of hyacinth bean as it enables harvesting of pods in several pickings ensuring continuous availability for a longer time. However, the market-led economy has necessitated production of hyacinth bean throughout the year and development of cultivars with synchronous pod-bearing ability to enable single harvest, which is possible only from photoperiod-insensitive cultivars (PIS) with a determinate growth habit (Keerthi et al. 2014b, 2016). Hence, major emphasis/objective of hyacinth bean breeding has been to develop PIS determinate cultivars. When using PIS cultivars, farmers can control the date of flowering, and hence maturity, simply by either varying the sowing date or choosing cultivars with different heat-unit requirements. However, selection for photoperiod insensitivity most often results in reduced vegetative phase, fewer braches, racemes and pods and hence reduced economic product yield. Although yields of such PIS varieties could be maximized by high-density planting (Shivashankar and Kulkarni 1989; Viswanath et al. 1971), developing PIS varieties with a minimum of 45 days from seedling emergence to early blooming would enable vegetative growth adequate enough to produce an acceptable economic product yield, even under normal density of planting as is practiced for PS cultivars (Keerthi et al. 2016).

Most of the improvement work on *typicus* and *lignosus* types is concentrated in India. Desired qualities in improved cultivars are high yield, short duration, determinate growth habit, day-length neutrality, uniform maturity and disease and pest resistance (Ramesh and Byregowda 2016). In Bangladesh, hyacinth bean breeding is being carried out in Mymensingh (Alam and Newaz 2005; Arifin et al. 2005). These programs are aimed at developing improved photoperiod insensitive determinate pureline varieties for year-round production of hyacinth bean for food use. On the other hand, in India at the Indian Grass Land and Fodder Research Institute (IGFRI) (Magoon et al. 1974) and in Australia (Whitbread and Pengelly 2004), hyacinth bean breeding programs are focused on developing pureline varieties for fodder use. In Australia, the strategy is to combine the traits of widespread forage variety Rongai with those of African wild perennial germplasm accessions (Whitbread and Pengelly 2004).

Development of pureline varieties is the major breeding option in hyacinth bean since it is a predominantly a self-pollinated crop (Chaudhury et al. 1989; Kukade and Tidke 2014) lacking pollination control systems. As in the case of other grain legumes, pedigree breeding is the preferred method of developing pureline varieties in hyacinth bean. Some varieties developed for food and fodder use in India, China, Australia and the USA are summarized in Tables 8.6 and 8.7 (Ramesh and Byregowda 2016).

Of the several biotic stresses, anthracnose and dolichos yellow mosaic virus (DVMV) diseases and pod borers (*Heliothis armigera* and *Adisura atkinsoni*) and bruchids (*Callosobruchus theobrome*), are major biotic production constraints in hyacinth bean (Ramesh and Byregowda 2016). While pod borers cause damage in the field, bruchids cause damage both in the field and in storage. Losses to pod borers and bruchids can be up to 100%. Breeding for resistance to these insect pests is currently limited to screening and identification of resistance sources in germplasm and breeding lines. Jagadeesh Babu et al. (2008) identified germplasm accessions such as GL 1, GL 24, GL 61, GL 69, GL 82, GL 89, GL 196, GL 121, GL 135, GL 412, and GL 413 with <10% insect damage as resistant to pod borers (*Heliothis armigera* and *Adisura atkinsoni*) and bruchids (*Callosobruchus chinensis*) based on field screening of 133 germplasm accessions. Based on laboratory screening of 28 selected germplasm accessions, Rajendra Prasad et al. (2013) identified resistant accessions, GL 77, GL 233 and GL 63 with least seed damages of 13.4, 14.69 and 18.34%, respectively. The germplasm accession GL 187 was identified as resistant to *Helicoverpa armigera, Adisura atkinsoni* and bruchids infestation (Rajendra Prasad 2015). In another study at UAS, Bengaluru, antixenosis and antibiosis mechanisms of resistance are highlighted against *Helicoverpa* and germplasm accessions GL 233, GL 426, GL 357 and GL 187 which were found moderately tolerant (Rajendra Prasad 2015).

Dolichos yellow mosaic virus (DYMV) is characterized by yellow to bright yellow patches and vein clearing on leaves (Maruthi et al. 2006); it is caused by the gemini virus and transmitted by whiteflies (Capoor and Verma 1950). The disease causes up to 80% crop loss (Muniyappa et al. 2003). As is the case with insect pests, breeding hyacinth bean for DYMV resistance is confined to identification of resis-

Table 8.6 Summary of hyacinth bean grain purpose varieties developed for commercial production

Varieties	Pedigree	Location	References
Hebbal Avare (HA) 1	Photoperiod sensitive local land race × photoperiod insensitive red *typicus*	University of Agricultural Sciences (UAS), Bengaluru, India	Viswanath et al. (1971)
HA 3	HA 1 × US 67–31 (an introduction from USDA	UAS, Bengaluru, India	Shivashankar et al. (1975)
HA 4	HA 3 × CO 8 (*typicus*, photoperiod insensitive	UAS, Bengaluru, India	Hiremath et al. (1979) and Mahadevu and Byregowda (2005)
Selections 1 & 2	Not reported	Indian Institute of Horticulture Research (IIHR), Bengaluru, India	Anon (1988)
Koala	Selection from accession introduced from France to Australia as Q 6680	Australia	Holland and Mullen (1995)
IPSA Seam -1 & IPSA Seam -2	Not reported	Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh	Rokhsana et al. (2006)
Xiangbiandou 1	Not reported	China	Peng et al. (2001)
RioVerde	Not reported	USA	Smith et al. (2008)

Source: Ramesh and Byregowda (2016)

Table 8.7 Summary of hyacinth bean forage varieties developed for commercial production

Varieties	Pedigree	Location	References
IGFRI -1- S- 2214 and IGFRI-1-S-2218	Not reported	IGFRI, India	Magoon et al. (1974)
Rongai	Pureline selection from germplasm accession, CPI 17883 introduced from Kenya	Australia	Wilson and Murtagh (1962)
Endurance	Rongai × African wild perennial germplasm accession, CPI 24973	Australia	Liu (1998)
Highworth	Pureline selection from germplasm accession, CPI 30212 introduced from south India to Australia	Australia	Liu (1998)

Source: Ramesh and Byregowda (2016)

tance sources. Singh et al. (2012) identified accessions VRSEM 894, VRSEM 887 and VRSEM 860 as resistance to DYMV among 300 germplasm accessions.

Hyacinth bean has better inherent capacity to withstand moisture stress than other legumes such as cowpea, horse gram, etc. (Ewansiha and Singh 2006; Maass et al. 2010; Nworgu and Ajayi 2005) and adapt to acidic (Mugwira and Haque 1993) and saline soils (Murphy and Colucci 1999). With its deep root system, hyacinth bean is not only drought tolerant (Cameron 1988; Hendricksen and Minson 1985; Kay 1979), but also has the ability to harvest soil minerals which are otherwise unavailable to annual crops (Schaaffhausen 1963). However, research on breeding hyacinth bean for resistance to abiotic stresses is limited. In the event of the imminent extremes of abiotic stresses driven by climate change, hyacinth bean would be a better alternative to more popular legumes. Thus, breeding and enhancing the economic value of hyacinth bean would provide a competitive edge to hyacinth bean producers. In this backdrop, hyacinth bean is regarded as one of the promising future crops for sustainable agricultural production.

8.10 Application of Genomic Tools

8.10.1 Molecular Genetic Diversity

The use of genomic tools such as DNA markers in hyacinth bean breeding is at an early stage due to their unavailability in large numbers. Nevertheless, independent marker systems based on sequence information such as RAPD and AFLP have been used to detect and characterize genetic variation among germplasm accessions and breeding lines. Literature on the use of DNA markers in analysis of genetic diversity is summarized in Table 8.8; it suggests the presence of ample variation in the gene pool of hyacinth bean.

DNA marker allele-based variation present in germplasm would be useful for determining whether morphometric traits-based variation reflect variations at DNA sequence level as well. It would also provide information on the population structure, allelic richness and parameters that specify diversity among germplasm to help breeders to choose the appropriate genetic resources for cultivar development more effectively. Most studies on genetic diversity analysis are based on RAPD and AFLP. However, the information obtained from these markers is not reliable due to poor reproducibility. Hence, sequence dependent simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) are highly preferred by researchers owing to their simple inheritance and amenability for automation and high reproducibility. SSR marker assay helps to understand genetic relationship among germplasm accessions/breeding lines, selection of parents for hybridization, organization of variation in germplasm accessions and identification of cultivars (Benabdelmouna et al. 2001).

Table 8.8 Summary of DNA marker-based genetic diversity analysis in hyacinth bean

Genetic material used	Marker used	References
CSIRO: 40 accessions	RAPD	Liu (1996)
Mapping population (cross of 2 CSIRO accessions)	RFLP, RAPD	Konduri et al. (2000)
Bangladesh/Japan germplasm +60 CSIRO accessions	RAPD	Sultana and Ozakiy (2000)
Mapping population for comparative mapping with mung bean (*Vigna radiata)*	RFLP	Humphry et al. (2002)
103 CSIRO accessions	AFLP	Maass et al. (2005)
11 varieties from Hunan province of China	RAPD	Tian et al. (2005)
12 landraces from southern India	RAPD	Gnanesh et al. (2006)
28 accessions + Tanzanian landraces	AFLP	Tefera (2006)
30 germplasm accessions of USDA	SSR	Wang et al. (2007)
62 landraces collected from southern India and core collection accessions	AFLP, SSR	Venkatesha et al. (2007)
47 accessions of USDA	SSR	Wang et al. (2007)
40 accessions from India	AFLP	Patil et al. (2009)
10 insect tolerant and susceptible landraces from India	RAPD	Sujithra et al. (2009)
Mapping population from cross of 2 Chinese accessions	RAPD	Yuan et al. (2009)
30 Indian accessions	RAPD	Rai et al. (2010)
22 accessions from India	AFLP	Venkatesh et al. (2010)
11 genotypes of hyacinth bean	RAPD	Biswas et al. (2012)
50 Kenya accessions	AFLP	Kimani et al. (2012)
13 Kenyan germplasm	SSR	Shivachi et al. (2012)
24 hyacinth bean accessions collected from China and Africa	EST-SSR	Guwen Zhang et al (2013)
36 hyacinth bean accessions	SSR	Laxmi et al. (2016)

8.10.2 Cross Legume Species/Genera Transferability of Markers

The use of transferable cross-species/genera SSR markers is an alternative strategy to ensure the availability of markers in genomic resource-limited crops such as hyacinth bean. Taking a clue from several successful examples of cross-transferability of SSR markers, Yao et al. (2012) demonstrated that all tested EST-SSR markers from soybean were cross-transferable to hyacinth bean. At the UAS, Bengaluru, transferability of SSR markers from cowpea, soybean, *Medicago truncatula,* green gram and chickpea to hyacinth bean were examined (Shivakumar and Ramesh 2015). Wang et al. (2004) also reported transferability of 1/3 (30.78%) of the SSR primers from *Medicago*, soybean, cowpea and groundnut to hyacinth bean. Venkatesh et al. (2007) examined the transferability of AFLP and EST-derived markers from a range of legumes to hyacinth beans collected from India, Australia

and Ethiopia. The results suggested that there is a good source of legume-related primers in databases from well-characterized species that can readily be used in diversity and genome analysis of hyacinth bean. Uday kumar et al. (2016) used 100 cross-legume species/genera SSR markers (65 from soybean, 12 from medicago, 14 from green gram, 9 from chickpea) to check parental polymorphism and found 18 of them (41.86%) were polymorphic between the parents of RILs. A total of 275 cross-legume species/genera SSR markers were examined for their transferability to hyacinth bean (Shivakumar et al. 2016). They found that 126 of 275 cross-legume species/genera SSR markers (45.81%) were transferable to hyacinth bean. The extent of transferability of SSR markers based on simple di−/tri-nucleotide repeat motifs was higher than those based on penta−/tetra−/complex nucleotide repeat motifs.

8.10.3 Mapping Genomic Regions Controlling Economically Important Traits

Conventional hyacinth bean breeding based on phenotype-based selection for yield and its component traits is rather less effective owing to their crop-stage specific expression, complex inheritance and significant cross-over genotype-by-environment interaction. DNA markers owing to their crop stage non-specificity, simple inheritance and environment neutrality have proven to be powerful surrogates of such difficult-to-select traits. Besides analysis of genetic diversity, DNA markers have also been used to develop a linkage map, a prelude to identifying DNA markers linked to genomic regions controlling target traits. DNA marker-assisted identification and introgression of QTLs into elite genetic background is expected to complement phenotype-based selection and help enhance the pace and efficiency of hyacinth bean breeding.

Konduri et al. (2000) were pioneers in the construction of a linkage map of hyacinth bean consisting of 127 RFLP and 91 RAPD loci in 119 F_2 population (Rongai× CPI 24973) of hyacinth bean. The map comprised 17 linkage groups (LG) and covered 1610.0 cM, with an average inter-marker distance of 7.0 cM. Later, Humphry et al. (2002) compared a linkage map of mung bean with hyacinth bean using a common set of 65 RFLP probes. A significantly high level of homology was noticed between mung bean and hyacinth bean.

In order to map the QTLs for various agronomic and phonological traits in hyacinth bean, Yuan et al. (2009) designed an F_2 population derived from the contrasting parents-Meidou 2012 and Nanhui 23. The molecular map was constructed with 131 loci (122 RAPD and 9 morphological markers) covering 1302.4 cM and 14 linkage groups. A total of 41 main effect QTLs (19 for fruit traits and 22 for growth phonological traits) were detected on 11 linkage groups. They also reported stable QTLs for pod length, pod diameter, pod fresh thickness, flowering time, podding time and harvest maturity period. Yuan et al.

(2011) also identified QTLs associated with various quantitative traits such as inflorescence length, peduncle length from branch to axil, peduncle length from axil to lowermost flowering node, rachis length, node number of inflorescence, rachis internode length, node order of the first inflorescence and node order of lowest inflorescence. In another study at UAS, Bangalore, 91 SSR markers out of 465 in-house developed hyacinth bean specific SSR markers were found to be polymorphic between the parents (HA 4 and CPI 60125) of HACPI 6-derived RIL population. The linkage map was constructed using genotypic data of 58 polymorphic markers in HACPI 6-derived 109 RIL populations; 58 markers were anchored on to 11 linkage groups (LGs). The total length of the map spanned 2008.55 cM of the hyacinth bean genome with an average marker density of 34.63 cM. The linkage map length varied from 118.77 cM (LG 10) to 261.06 cM (LG 4). A total of 5 QTLs, 1 controlling days to 50% flowering, 2 each controlling dry seed yield plant^{-1} and test weight were detected (Chandrakant 2018). Furthermore, the linkage of markers with QTLs controlling days to 50% flowering; raceme length; pods plant^{-1} and dry seed yield plant^{-1} in HACPI 6-derived RIL population was confirmed in HACPI 3-derived RIL population. However, it is suggested to saturate the linkage map of HACPI 6-derived RIL population for high-resolution mapping of QTLs controlling productivity per se traits for use in marker-assisted selection after their validation (Chandrakant 2018).

Association mapping (AM) is an alternative method of QTL discovery which exploits historic linkage disequilibrium (LD) present in natural populations. AM is effective in self-pollinated crops such as hyacinth bean as LD extends over a longer genomic distance driven by a low rate of recombination and thereby requiring fewer markers for exploring marker-trait associations. Vaijayanthi (2016) evaluated a core set of hyacinth bean germplasm consisting of 64 accessions for 9 quantitative traits (QTs) and genotyped it using 234 SSR markers. Substantial diversity was observed among the core set accessions at loci controlling QTs and 95 of the 234 SSR markers were found to be polymorphic. The structure analysis based on 95 polymorphic SSR markers revealed weak population structure, low magnitude of the estimates of fixation indices, which in turn indicated low possibility of false discovery rates in marker-QTs association. The marker alleles' scores were further regressed onto phenotypes at 9 QTs following general linear model (GLM) and mixed linear model (MLM) for exploring marker-QTs associations. A few of the significantly associated markers such as KTD 200 for days to 50% flowering, KTD 273 for fresh pod yield plant^{-1} and KTD 130 for fresh pods plant^{-1} explained $\geq$10% of the trait variations. These linked SSR markers are suggested for validation for their use in marker-assisted hyacinth bean improvement programs.

Marker-assisted selection (MAS) is most effective for improvement of traits controlled by a few large effect genes. QTs are controlled by both large and small effect QTLs. Genomic selection (GS), proposed by Meuwissen et al. (2001), captures both small and large effects QTLs (Bernardo and Yu 2007; Bernardo 2010) and is emerg-

ing as a powerful alternative to MAS for improving QTs. GS is defined as the selection of a genotyped-only breeding population (BP) of individuals based on their genomic breeding values (GBVs) predicted using marker effects estimated by fitting statistical models calibrated to both genotyped and phenotyped populations referred to as a training population (TP), preferably related to a breeding population (BP). Recently GS was attempted in hyacinth bean (Chandrakant 2018). A total of 109 RILs derived from HACPI-6 were used as the training population (TP). The 2 year phenotypic data (BLUPs) and genotypic data (91 SSR markers) of TP were used to calibrate the ridge regression (RR) to estimate the effects of 91 SSR markers. The study necessitated an optimizing prediction model, composition and size of the training population and marker density for implementing genomic selection in hyacinth bean.

Application of modern crop improvement techniques like plant tissue culture, genetic engineering and omic-driven technologies are in their infancy in hyacinth bean. However, genetic manipulation based on such modern techniques can add a competitive edge and direction to selective breeding programs to evolve better hyacinth bean varieties.

8.11 Conclusion and Prospects

Despite being a multipurpose adaptable legume crop, hyacinth bean is considered an underutilized crop owing to the small area under cultivation and limited efforts towards its genetic improvement. However, it can contribute enormously to food security and better nutrition, ecosystem stability and cultural diversity. It is often called the *poor-man's* bean, a kind of confirmation of its low-input production and an essential contribution to the human diet in certain regions. Conservation and utilization of plant genetic resources is the key to attain sustainable hyacinth bean productivity and production. Systematic evaluation of germplasm resources, identification of trait-specific accessions, unraveling the inheritance of productivity traits and the use of both conventional and genomic tools to combine desired traits will provide competency in hyacinth bean improvement programs. The SSR and SNP markers should be routinely used for genomic selection to complement phenotype-based selection. Furthermore, genome sequencing and other omic approaches help to identify novel and useful genes and their introgression into an elite agronomic background.

Appendices

Appendix 1: List of Major Institutes Engaged in Research on Hyacinth Bean

Institution	Specialization and research activities	Website
University of Agricultural Sciences (UAS), Bengaluru, India	Germplasm collection, Conservation and utilization	www.lablab.org
	Conventional and marker-assisted hyacinth bean improvement	www.uasbangalore.edu.in
	Breeding for pod borer resistance	
CSIRO, Australia	Breeding hyacinth bean for forage purpose	www.csiro.au
IITA, Nigeria	Germplasm collection, Conservation and utilization	www.iita.org
	Breeding for biotic and abiotic stress	
Bangladesh Agriculture Research Institute (BARI), Bangladesh	Hyacinth bean improvement using conventional and molecular tools	www.bari.gov.bd
Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India	Breeding hyacinth bean for vegetable purpose	www.icar.org.in
		www.iivr.org.in
Indian Institute of Horticulture Research (IIHR), Hesarugatta, Bangalore, India	Improvement of *Lablab purpureus* var. *typicus* and of *L. purpureus* var. *lignosus*	www.iihr.res.in
		www.icar.org.in
Tamil Nadu Agriculture University, India	Hyacinth bean improvement using conventional and molecular tools	www.tnau.ac.in
Kenya Agriculture Research Institute, Kenya, Africa	Germplasm collection, evaluation and breeding for African countries	www.kalro.org
		www.ilri.org
USDA-ARS,USA	Hyacinth bean conservation and improvement	www.ars.usda.gov

Appendix 2: Genetic Resources of Hyacinth Bean

Cultivar	Cultivation location
HA-1, HA-3, HA-4,Co-1, Co-2, Arka-Vijay, Kalyanpur type-2, Jawahar Sem-37, Deepali, Wal Konkan-1, Hima, Grace, Pusa sem1 & 2	India
CPI 30212 (High worth), Rongai, CPI 24973 (Endurance)	Australia
Local varieties and landraces	Bangladesh
Amora-guaya, Gerenga, Njahe	Africa

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Chapter 9
Lentil (*Lens culinaris* Medik.) Diversity, Cytogenetics and Breeding

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Abstract Lentil (*Lens culinaris* Medik. ssp. *culinaris*) is one of the oldest cultivated plants that originated from *L. culinaris* Medik.ssp. *orientalis* in the Near East arc and Asia Minor. This cool season legume crop is an excellent food source to provide energy, proteins and iron in the human diet. Most lentil-growing countries have a shared objective of higher and more stable seed yield, which often entails breeding for adaptation to abiotic and biotic stresses, which otherwise cause a substantial reduction in crop yield and production. Lentil domestication and selection over thousands of years led to the low amount of genetic variation in the current cultivated species and this scarcity in genetic variability represents a major constraint for lentil breeding. Thus far, lentil breeders have been successful in improving some easily manageable monogenic traits using conventional breeding techniques of selection and recombination. However, these conventional techniques are insufficient to address economic traits like seed yield due to polygenic inheritance and genotype-environment interaction. Other species of the genus *Lens* are important sources of genetic variation for breeding key traits into new lentil varieties. Induced mutagenesis is a powerful breeding tool and can greatly supplement the availability of lentil genomic resources. Impressive progress in applications of biotechnological innova-

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tions in the utilization of genetic resources for lentil genetic improvement will further accelerate the development of improved varieties. This chapter provides an overview on present status of lentil genetic improvement and summarizes the various important aspects of lentil diversity, cytogenetic and breeding.

Keywords Biodiversity · Cytogenetics · Genomic tools · Lentil · Micromutations · Mutation breeding · Stress biology

9.1 Introduction

The reduced per capita availability of food due to the unprecedented rate of human population growth urgently demands the attainment of enhanced productivity, especially in developing countries like India, Pakistan and Bangladesh. According to the Food and Agriculture Organization of the United Nations (FAO) estimates, the world agricultural production will experience an unprecedented confluence of around 70% more demand to feed the increasing global population expecting to reach over 9 billion by 2050, with the largest share from South Asia and Africa. The stagnant or dwindling state of arable land and water resources due to additional urbanization and industrialization demands, especially in the critically food insecure regions of the developing world, compounds the constrictions to achieve the target of accelerating food production (FAO 2009). Since, neither the exploitation of additional land and water resources is recommendable, nor the increased unsustainable use of agrochemicals; therefore, production of more food with fewer agricultural inputs is the only practicable option in the present global scenario (Mba 2013). As per the recommendation of FAO (2011) for the establishment of low-input agriculture in the twenty-first century, it is imperative to generate improved crop varieties that are genetically diverse, climate-change resilient, input use-efficient, high yielding, have enhanced nutritional attributes and widely adaptable to a range of agroecosystems and farming practices. Therefore, the sustainable intensification of the agricultural production through low-input agriculture by developing high-yielding varieties of major food crops is the only solution to combat the global food crisis. Plant breeding strategies for the development of the FAO recommended genetically diverse suite of crop varieties requires broad genetic variations as an essential initial input, thus the availability of accessible genetic variability is highly desirable to the plant breeders and geneticists. The extremely narrow genetic base of the available crop varieties is the major constraint to breeding desirable crop varieties, that are suitable for enhancing the farm productivity and establishing food security. The genetic bottleneck conditions of major food crops such as cereals and pulses, develops over the years due to the adaptation to various stresses through natural selection and unidirectional conventional selection methods for yield traits, resulting in limited accessible genetic variability to the breeders for further improvement in the crops. The available genetic variability in domesticated crops, especially grain legumes, has been exhausted, which necessitates the induction of innovative breeding tools for generating new genetic variability in the yield traits. Mba et al. (2012) advocated the advantage of

induced mutagenesis to unleash new alleles of genes that control the traits desired for elite crop varieties of the twenty-first century.

Lentil, being a climate resilient and highly nutritious pulse crop, fits extremely well in the target food crops to be improved into genre of a *smart crop* and, therefore, proper scientific investment in the lentil genetic improvement is required. Khazaei et al. (2016) reported that Canadian and South Asian lentil germplasm mostly has a narrow genetic base because the yield of lentil is under constant threat in these regions from biotic and abiotic stresses, in the context of the already climate-threatened food security crisis. This highlights the necessity of induction of mutations in desirable gene(s) responsible for yield trait for development of high yielding lentil cultivars with wide adaptability. Erskine et al. (1998) and Toker et al. (2007) reviewed the different plant breeding methods utilized in various self-pollinated crops and principally recommended mutation breeding as an extremely important technique to broaden the genetic base of lentil under the present bottleneck conditions. Recent advancements in modern genomic techniques also have significant impact on the utility of mutation breeding in lentil genetic improvement.

9.1.1 Botanical Classification and Distribution

As a preferred food legume, lentil is known by as many as 30 different common names around the world (Kay 1979) including *masser* (India), das (Arabic), *mercimek* (Turkey), *messer* (Ethiopia), and *heramame* (Japanese), to name a few (Muehlbauer and Mcphee 2005). The genus *Lens* Mill. is a relatively small genus of the legume tribe Vicieae that includes four other genera: *Vicia* L., *Lathyrus* L., *Pisum* L. and *Vavilovia* Al. Fed. (Redden et al. 2007). *Lens culinaris* Medik. is the only cultivated species of the genus *Lens* (Ferguson 2000). In 1787, the German botanist Medikus assigned lentil the scientific name *Lens culinaris* (Fratini et al. 2014).

The present taxonomic status of lentil may be stated as follows, based on the USDA, Plants Database 2017:

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Sub-kingdom	Tracheobionta – Vascular plants
Super-division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae/Leguminosae – Pea family
Genus	*Lens* Mill. – Lentil
Species	*Lens culinaris* Medik. – Lentil

According to Muehlbauer et al. (1980), the taxonomic position of the genus *Lens* is between *Vicia* and *Lathyrus*, relatively closer to *Vicia* within the subfamily Papilionaceae of the family Fabaceae. According to the latest classification, the genus *Lens* consists of seven taxa in four species (Ferguson and Erskine 2001; Ferguson et al. 2000).

Lens culinaris Medik.

ssp. *culinaris*
ssp. *orientalis* (Boiss.) Ponert
ssp. *tomentosus* (Ladiz.) M.E. Ferguson& al.
ssp. *odemensis* (Ladiz.) M.E. Ferguson& al.

Lens ervoides (Brign.) Grande
Lens nigricans (M. Bieb.) Godron
Lens lamottei Czefr.

Lentil is an annual herb, erect in growth form, much branched with a slender stem; leaves are alternate, compound, pinnate, usually ending in a tendril or bristly; leaflets alternate or opposite, stipules small and linear. Flowers are small, pale blue or purple, 1–4 flowers in axillary racemes. Pods are oblong, flattened or compressed, smooth containing 1–2 seeds; seeds lens shaped; greenish brown or light-red. The hypogeal germination found in lentil allows developing seedlings to grow below the ground level, which facilitates evading harsh environmental conditions at an early growing stage (Muehlbauer et al. 1985). Self-pollination is a common phenomenon in lentil, with cross-pollination reported usually <1–6.6% (Baum et al. 1997). Flowering takes place acropetally (upward).

Cubero (1981) subdivided *Lens culinaris* on the basis of seed size into two races: *macrosperma* and *microsperma*. The distinguishing characteristics of the two races (Cubero et al. 2009) are as follows:

Macrosperma Large pods (15–20 × 7.5–10.5 mm) generally flat enclosing large flattened seeds; 1000 seed weight is 25–50 g. Cotyledons yellow or orange. Flowers large (7–8 mm long), white with veins, rarely light blue, very light or no pigmentation, 2–3 flowered peduncles. Long calyx teeth. Oval shaped large leaflets with dimensions 15–27 × 4–10 mm, length to width ratio range is 3–3.5. Height of plant is 25–75 cm.

Microsperma Pods small or medium (6–15 × 3.5–7 mm), convex. Seed f1attened – subg1obose, small or medium (3–6 mm diameter) with 1000 seeds weight up to 25 g. Cotyledons red, orange or yellow. Flowers white to violet, small (5–7 mm long),and 1–4 flowered peduncles. Elongated linear or 1anceo1ate shaped small leaflets with dimensions 8–15 × 2–5 mm, length to width ratio is 4–5. Height of plant 15–35 cm.

9.1.2 History, Origin and Domestication

Lentil was domesticated in the Fertile Crescent of the Near East and then spread to Europe, the Middle East, Northern Africa and the Indo-Gangetic plain (Ford et al. 2007). The primary center of diversity for the domestic *Lens culinaris* as well as its wild progenitor *L. culinaris* ssp. *orientalis* is considered to be the Middle East (Cubero 1981; Zohary 1972). It is believed to be as old a crop as einkorn, emmer barley and pea (Harlan 1992). Dhuppar et al. (2012) considered lentil to be one of the earliest domesticated pulses and grown for some 8000 years ago. It is indigenous to southwestern Asia and Mediterranean region (Cokkizgin and Shtaya 2013). It is now cultivated in most subtropical and temperate areas, and more particularly where rainfall is low. Khazaei et al. (2016) reported that global cultivated lentil germplasm clustered, primarily based on eco-geographical origin, into three basic groups: subtropical savannah, Mediterranean and northern temperate. Lentil cultivars are often divided into two cultivar groups. Europe, North Africa and America are known for cultivation of mostly large seeded *macrosperma* group, whereas the small seeded *microsperma* group is cultivated mainly in Asia, Egypt and Ethiopia. In western Asia and southeastern Europe both cultivar groups are grown (Ford et al. 2007).

Helbeck (1963) stated that the history of lentil is ancient, as old as agriculture itself. The oldest known carbonized remains of lentil from Greece's Franchthi cave are dated to 11,000 BC (Sandhu and Singh 2009). Lentil was an important part of the initial agricultural revolution in the Old World (Zohary 1976). Although, the Congress on Botanical Nomenclature in 1966 credited the name *Lens* to Miller to resolve author confusion, the pre-Linnaean French botanist Tournefort is considered to be the first person to use the name *Lens*. In 1741, Miller classified lentil into only three species: *vulgaris*, common lentils; *major*, greater lentils and *monanthos*, lentils with a single flower. In archaeobotanical excavations carbonized remains of lentil seeds have been recovered from widely dispersed places such as Tell Ramand in Syria (6250–5950 BC), Aceramic Beidha in Jordan, Hacilar in Turkey (5800–5000 BC) and Tepe Sabz in Iran (5500–5000 BC) (Helbeck 1970; Van Zeist and Bottema 1971), Argissa-Magula Tessaly in Greece (6000–5000 BC) (Hopf 1962), Nea Mikomedeia, Macedonia in Greece (6000–5000 BC) (Renfrew 1969; Van Zeist and Bottema 1971), Matmur, El Omari in Egypt (Helbeck 1963) and the Harappan Civilization in India (3300–1300 BC) (Cubero et al. 2009).

The reviews of archaeological studies presented above, along with Ford et al. (2007) substantiates the presence of lentil cultivation as far back as 8500–600 BC in the present-day region of Turkey, Syria and Iraq. Lentil domestication began in this region and spread to the Nile Valley, Greece, Central Europe and eastwards to South Asia (Nene 2006). It later dispersed to Ethiopia, Afghanistan, India, Pakistan, China and Latin America (Cubero 1981; Duke 1981; Ladizinsky 1979). Erskine et al. (1989) stated that lentils were cultivated along with wheat and barley from the Bronze Age onward throughout the expanding realm of Mediterranean-type agriculture. Considering the fact that lentil yield is relatively lower than that of wheat and barley, the subsequent cultivation of lentil with them suggests that demand for

lentils existed in the social structure of that time. Thus, lentil cultivation, together with other grain crops, is considered to be a part of late fifth or early fourth millennium BC farming systems (Sandhu and Singh 2009). Archaeobotanical remains indicate that the domestication of lentil began prior to 2500 BC in India (Sandhu and Singh 2009). According to Vishnumittrre (1974), lentil was domesticated in India in the Neolithic Chalcolithic period. Cubero et al. (2009) demonstrated that *Lens orientalis* is the progenitor of the cultivated lentil *L. culinaris*, based on the cytogenetic analysis of relative chromosomal interchanges in the interspecific hybrids of *L. culinaris* with *L. nigricans* and *L. orientalis.*

9.1.3 Cytogenetics

Lentil cultivars and their wild relatives are self-fertilizing diploids with a chromosome number 2n = 2x = 14. Even after several attempts to account for the standard karyotype of lentil, contradictory results have been reported by different researchers (Sharma 2009). Total chromosome length is reported as 39.31 μm in variety Pant L 639 (Gupta and Singh 1981), 31.77 μm in Type 36(Dixit and Dubey 1986), 28.2–72.3 μm in different *microsperma* group cultivars of *Lens culinaris* ssp. *culinaris* (Dixit and Dubey 1986); 21.47 μm in *L. ervoides* and 30.4 μm in *L. orientalis* (Sindhu et al. 1983). Lavania and Lavania (1983) reported the lowest chromatin length of 16.9 μm. Mishra et al. (2007) considered that the length of chromosomes in lentil had a range of 3.0–9.2 μ. The structure of chromosomes in lentil has also been shown to vary by species and among genotypes of the same species. Sindhu et al. (1983) presented the karyotype of lentil as the somatic tissues of *L. culinaris*, *L. orientalis* and *L. nigricans* includes three sub-metacentric (one of them with a secondary constriction), one metacentric and three acrocentric chromosome pairs. It was also noticed that the karyotype of *L. orientalis* was similar to *L. culinaris* (Ladizinsky 1979; Ladizinsky et al. 1984) and *L. culinaris* differed from *L. nigricans* by three interchanges (Gupta and Bahl 1983).

Harlan and de Wet (1971), based their results of hybridization among species, proposed three gene pool concepts, namely primary (GP-1), secondary (GP-2) and tertiary (GP-3). The gene transfer between GP-1 and GP-3 is very difficult and the crosses between them results in anomalous, lethal and completely sterile F_1 progenies. The gene transfer between GP-1 and GP-2 is comparatively possible with some fertility in F_1 progenies. Crossing within GP-1, which consists of landraces and biological species, is easy and F_1 hybrids are vigorous, exhibiting normal meiotic chromosome pairing with total fertility. The GP-1 gene pool was further subdivided into GP-1 (A): cultivated races and landraces and GP-1 (B): subspecies, wild and weedy relatives. Ladizinsky (1999) based on crossability assigned *Lens* species into primary, secondary, and tertiary gene pools. GP-1: *Lens culinaris* ssp. *culinaris, orientalis, odemensis*; GP-2: *L. ervoides, L. nigricans;* GP-3; *L. lamottei, L. culinaris* ssp. *tomentosus. L. culinaris* ssp. *orientalis* exhibited resistance to cold,

drought, wilt and *Aschochyta* blight. *Lens nigricans* and *L. culinaris* can form hybrids but with low seed set (Redden et al. 2007).

Although the chromosome number of 2n=2x=14 is common in all *Lens* species, differences in the chromosome arrangements are considered to be the primary reason behind the reproductive isolation between species(Muehlbauer and McPhee 2005). Recent work of Gaffarzadeh-Namazi et al. (2007) showed that the cultivated *L. culinaris* karyotype exhibits four pairs of metacentric and three pairs of submetacentric chromosomes with secondary constriction near centromeric region of long arm of chromosome 4. The research also revealed that considerable polymorphisms are seen in C-banding patterns among genotypes of lentil while there are minor differences in chromosome morphology. Lentil has a relatively large genome size of 4063 Mbp/1C as determined by flow cytometry(Arumuganathan and Earle 1991). The genetic linkage map of lentil is still quite rudimentary.

9.2 Nutritional Values and Importance

Lentil is considered to be among the first foods cultivated and is considered an important food since ancient times (Sarker and Erskine 2006). Lentil is known by many names in different countries and languages, indicating the breadth of its cultivation and importance. The dietary importance of lentils as essential macro- and micronutrients sources has been recognized since earlier times for human welfare.

Pulses provide important nutrients for humans and also for animals. They provide protein, complex carbohydrates, dietary fiber, vitamins and dietary minerals. Up to 25% of protein by weight is contained in pulses, double the amount present in wheat and three times that of rice. Lentils are highly nutritious and an easily digestible pulse crop. Lentil contains protein, vitamin A, fiber, starch, potassium, B vitamins and iron. The insignificant level of cholesterol, fat and antinutrients found in lentil is attributed to it being the most desired protein source for human consumption. (Sultana and Ghafoor 2008). The nutritional value of lentil is presented in Table 9.1. The amino acid profile of lentil protein is deficient in the sulfur-containing amino acids methionine and cystine, and in tryptophan (Table 9.2). It also serves a good source of fiber. Lentils cook quickly hence there is less nutrient loss. Different lentil recipes are used throughout South Asia. Lentil curry *dhal* is part of the everyday diet in the Indian subcontinent and can be cooked together with rice. *Mejadra* is a lentil and rice dish in Arab countries also known as *mujaddra*. *Kichdi* is also prepared from rice and lentil. Lentil flour can be used to make soups, stews and purees and mixed with cereals to make bread and cake.

Studies on human nutrition have shown that lentil consumption is healthy and eases oxidative stress, improves serum antioxidant capacity and reduces concentrations of total and low-density lipoprotein-cholesterol, triglycerides, adhesion molecules and inflammatory biomarkers that constrain the progress of cardiovascular diseases (Azadbakht et al. 2007; Crujeiras et al. 2007; Esmaillzadeh and Azadbakht

Table 9.1 Nutritional value of lentil, raw (dry weight) per 100 g (3.5 oz) (Nutrient values and weights are for edible portion)

Nutrient	Value
Water	10.40 g
Energy	343 kcal
Protein	25.80 g
Total lipid (fat)	1.06 g
Carbohydrate	60.08 g
Fiber, total dietary	30.50 g
Sugars, total	2.03 g
Minerals	
Calcium, Ca	56 mg
Iron, Fe	7.54 mg
Magnesium, Mg	122 mg
Phosphorus, P	451 mg
Potassium, K	955 mg
Sodium, Na	6.00 mg
Zinc, Zn	4.78 mg
Vitamins	
Vitamin C, total ascorbic acid	4.40 mg
Thiamin	0.873 mg
Riboflavin	0.211 mg
Niacin	2.605 mg
Vitamin B-6	0.540 mg
Folate, DFE	479 µg
Vitamin A, RAE	2.00 µg
Vitamin A, IU	39 IU
Vitamin E (alpha-tocopherol)	0.49 mg
Vitamin K (phylloquinone)	5.0 µg
Lipids	
Fatty acids, total saturated	0.156 g
Fatty acids, total monounsaturated	0.189 g
Fatty acids, total polyunsaturated	0.516 g

Source: USDA, National Nutrient Database for Standard Reference 2018

2012; Taku et al. 2007). The United States Department of Agriculture (USDA 2010) database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods shows that lentils have a relatively higher ORAC value (7282) than many other common food items included in different categories. However, in 2012, the Nutrient Data Laboratory (NDL) of USDA removed the ORAC table from the website due to the development that the values of antioxidant molecules in the food have multiple functions, therefore, not necessarily correlated directly to the ability to absorb free radicals in humans and it was done also to restrict the misuse of ORAC values by the food and supplement manufacturing companies in product promotions (https://www.ars.usda.gov).

Table 9.2 Amino acid composition of lentil

Amino Acid	Value g per 100 g
Protein	24.63
Alanine	1.029
Arginine	1.903
Aspartic acid	2.725
Cystine	0.322
Glutamic acid	3.819
Glycine	1.002
Histidine	0.693
Isoleucine	1.065
Leucine	1.786
Lysine	1.720
Methionine	0.210
Phenylalanine	1.215
Proline	1.029
Serine	1.136
Threonine	0.882
Tryptophan	0.221
Tyrosine	0.658
Valine	1.223

Source: https://www.nutritionvalue.org/Lentils%2C_raw_nutritional_value.html

Pulses are good sources of phenolic compounds such as flavonoids, isoflavones and phenolic acids. Additionally, the potential application of lentil protein hydrolysates as hypotensive ingredients containing angiotensin I-converting enzyme inhibitory peptides have been shown (Barbana and Boye 2011; Boye et al. 2010). Therefore, lentils could be considered as a valuable source of cardioprotective compounds. Lentil seeds are used to treat diabetes especially, Type II (Rizkalla et al. 2002) and are topically applied for the treatment of skin infections and burns as traditional medicine (Takruri and Issa 2013). Lentil possesses protein said to contribute to the lowering of blood pressure (Faris et al. 2013). Shams et al. (2008) reported that there is a significant decrease in fasting blood glucose when 50g of cooked lentil are added to a diabetic patient's diet.

9.3 Adaptation and Cultivation

Lentils are suitable for cultivation in warm temperate, subtropical and high altitude tropical regions of the world (Muehlbauer et al. 1995). They are mostly grown alone; however. intercropping with other crops like wheat, rice, barley, mustard, sugarcane, castor bean and linseed can be done (Andrews and Mckenzie

2007), and is recommended for increasing land outputs. The International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, maintains the world's largest collection of lentil genotypes (>10,000) (Sarker et al. 2002). Drought (usually linked with high temperature), low temperature and disease are major constraints hampering lentil yield lentil globally, although other local factors such as salt stress, nutrient deficiency and nutrient toxicity can also significantly influence yield (Muehlbauer et al. 2006; Tivoli et al. 2006). Lentils have an in-built competence to survive moisture stress as well as problematic soil conditions, along with high yield potential that imparts a very wide adaptability to this crop (Mishra et al. 2007).

The agroclimatic conditions and cropping systems in which lentil is cultivated vary across growing regions. Lentils are grown as a winter crop in subtropical areas and a summer crop in temperate countries. In the tropics it can be cultivated at higher altitudes (> 1800 m) during the cool season (Bejiga 2006). In the subtropics, including the Indian subcontinent, lentils are sown under moderately high temperatures when the rainy season ends and harvested in the dry season, at the beginning of summer. Variations in cropping system and crop season differ from region to region and significantly influence lentil productivity. Lentils do well with annual rainfall of about 750 mm with a marked dry period before harvest; some cultivars can sustain drought periods. Vegetative growth is luxuriant due to high humidity and excessive rainfall. Lentil plants exposed to drought or high temperature during flowering and pod filling stages generally suffer yield loss, while heavy textured soil is another cause for reduced yield. Sandy loam soils are best suited for lentils(Ozdemir 2002; Sehirali 1998) with high phosphorus content for better yield. Soil pH of 6–8 is best for lentil growth. The nitrogen requirement is fulfilled by inoculation of rhizobium. About 33–45 kg/ha of rhizobium is required by the young plants only until the development can affect/inhibit the nodulation and can lead to excessive vegetative growth and this excessive vegetative growth in turns reduces seed yield. Phosphorus and potassium are also applied and are believed to increase yield. Fertilizer application depends upon the soil type and is added only after soil analysis. Weeding should be done between planting and also during the growing season; it can be carried out by either mechanical or chemical means, and also by traditional hand weeding in small fields. Mechanical weeding is carried out either by rotary hoeing or by harrowing. Quackgrass can be controlled by the application of glyphosate. For 10–15 cm tall grasses, Poast (sethoxydim) treatment is given. Crop rotation is an effective method of preventing diseases like *Ascochyta* blight, *Sclerotinia* (white mold), *Fusarium* root rot and *Rhizoctonia*. Harvesting is usually done when plants begin to turn yellow and the lower pods turn brown to yellow brown in color. Lentils are allowed to dry in the field or in air dryers at a temperature of 43 °C.

9.4 Production Statistics

Cultivation of lentil (*Lens culinaris* ssp. *culinaris*) in South Asia dates back to around 2000 BC. About one-half of the world's lentils are grown in South Asia, and it is the main pulse crop in the region due its high nutritive value and soil ameliorative properties. The adaptation and cultivation of improved lentil varieties have contributed to food and nutritional security with increased yield, total production and farmer's income in South Asia.

Lentil (*Lens culinaris* Medik), was domesticated concurrently with wheat and barley in the Fertile Crescent, from today's Jordan northward to Turkey and southeast to Iran. Still today, a considerable part of global lentil production is concentrated in these regions; however, India and Canada are the largest producers of lentils. India is the world's largest producer and consumer of pulses accounting about 35% of the world area, about 26% of the total production with a yield gap of about 18% and about 30% of the total consumption in the world (Laskar et al. 2018a). On the basis of total global production, lentil ranks sixth after dry bean, pea, chickpea, faba bean and cowpea. Asia has the highest share of 46.2% lentil produced worldwide in the last decade followed by 44.5% by the Americas (Fig. 9.1). India, Bangladesh, Myanmar, Nepal and Pakistan in the South Asian region are the leading contributors. India and Canada are the leaders in their respective regions; however, Canada is increasing its production share in the global market comparably at a significantly higher rate than India. Although area under lentil cultivation is considerably high in India, Canada is comfortably ahead of India in total production due to their high yield.

Recent lentil production statistics rank India second in both area and production with 28.24% and 16.71% of world area and production, respectively (Table 9.3). This reflects the continuous efforts of lentil cultivation in Canada leading to a significant increase in their area under cultivation over the years and supplemented with high-yielding cultivars and modern management practices, Canada is far way ahead of all other countries including India in production. One of the drawbacks in South Asia is the low yield of cultivated lentil varieties. Lentil yield in India is

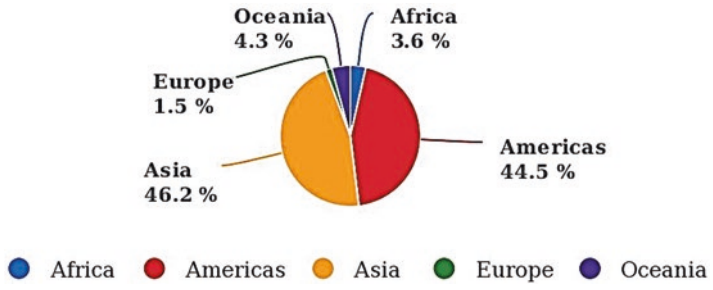

Fig. 9.1 Production share of Lentils by region (average 2006–2016). (Source: FAOSTAT, FAO Statistics Division 2018)

Table 9.3 Global ranking of major countries in area, production and yield of lentil

Rank	Countries	Area harvested (ha)	World avg. (%)	Countries	Yield (kg/ha)	World avg. (+)	Countries	Production (mt)	World avg. (%)
01	Canada	2,175,200	39.69	New Zealand	2590	1438	Canada	3,233,800	51.20
02	India	1,548,106	28.24	China	2374	1222	India	1,055,536	16.71
03	Turkey	246,322	4.49	Egypt	2002	850	Turkey	365,000	5.78
04	Australia	224,944	4.10	Armenia	2000	848	USA	255,061	4.04
05	Nepal	205,939	3.76	Canada	1487	335	Nepal	253,041	4.01
06	USA	186,079	3.39	Turkey	1482	330	Australia	181,638	2.88
07	Bangladesh	154,515	2.82	Ethiopia	1463	311	Ethiopia	166,274	2.63
08	Syria	130,286	2.38	Argentina	1444	292	Bangladesh	158,228	2.51
09	Iran	129,521	2.36	USA	1371	219	China	142,991	2.26
10	Ethiopia	113,685	2.07	France	1323	171	Syria	112,193	1.78

Source: FAOSTAT, FAO Statistics Division 2018

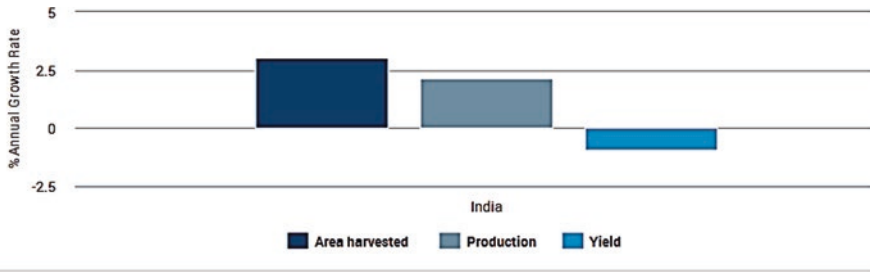

Fig. 9.2 Annual growth rate of lentil in India (2005–2014). (Source: FAOSTAT, FAO Statistics Division 2017)

almost half (682 kg/ha) of the world average (1152 kg/ha), which is the principal constraint in this region. The highest productivity is recorded in New Zealand (2590 kg/ha) followed by China (2374 kg/ha). Apparently the inefficient agricultural practices and low yielding cultivars are the constraining factor in the less productive regions, including India. Annual growth rate estimation in the period 2006–2018 in India showed a 3.06% and 2.13% increase in total area harvested and total production, respectively, but a decrease of 0.91% in yield (Fig. 9.2). This evidences that despite sustained efforts from government and plant breeders, the low yield of lentil cultivars remains the major obstacle in achieving higher production goal.

9.5 Production Constraints

The inherent potential of pulse crops for genetic improvement of yield traits is greatly restricted by biotic and abiotic factors (Tomlekova 1998; Yankova and Sovkova-Bobcheva 2009). Lentil landraces typically exhibit limited yield potential with considerable vulnerability towards different stresses, which adversely affects average lentil yield. Different biotic and abiotic stresses along with other local factors hamper the yield of lentil globally, especially in resource-poor areas (Muehlbauer et al. 2006; Sinclair and Vadez 2012; Tivoli et al. 2006). Biotic stresses such as the pod borer, aphids, cutworms, powdery mildew, rust and wilt are major pests and diseases affecting lentil production. Legumes are rich in nitrogen and phosphorus due to their ability to fix atmospheric nitrogen; this property attracts pests and diseases (Sinclair and Vadez 2012). Abiotic limiting factors include drought, temperature stress like high temperatures during pod filling and cold temperatures in winter (Silim et al. 1993a). Agronomic problems of pod dehiscence, lodging and inadequate crop management are additional constraints.

South Asian countries, led by India, are contributing almost one-half of the world lentil production; however, owing to the high demand for lentils, these same countries are also among the highest importers of lentils. The indigenous lentils in this region are of a specific ecotype (Pilosae) which is an endemic group with narrow genetic variability. The lack of marked variability for morphological, phenological and yield-related traits and resistance to key stresses have handicapped lentil improvement programs for long time in the region. The introduction of cv. Precoz, a bold-seeded plant of Argentine origin, and other exotic germplasms for use in hybridization programs in the region, have attempted to broader the narrow genetic base (Erskine et al. 1998). The hybridization programs with diverse crosses between germplasm of different origins have generated enormous variability for agro-economic traits of interest like seed traits, growth habit, plant height, crop duration, biomass production, tolerance to drought, rust, blight resistance, etc. The selection of promising progenies has led to the development of improved lentil varieties in Afghanistan, Bangladesh, India, Nepal and Pakistan. A diverse genetic background facilitated these improved cultivars to be adapted in new agro-ecological zones according to climate change, consumer preference and market demand.

India is the largest producer of pulses but farmers give second priority to pulses after cereals and cash crops because of low purchasing power and accessibility to markets to sell the excess produce. Good quality seeds and other inputs are major constraints in increasing the production of grain legumes (David et al. 2002). In India, a yield gap of 30–105%, with an average 42%, has been reported from different locations (Reddy and Reddy 2010), due to low temperatures at seedling and flowering stages, intermittent or terminal drought and heat stress during crop cultivation (Farooq et al. 2009; Silim et al. 1993b; Wery et al. 1994). Improvement of different pulse varieties is likely to increase the yield by 20–25%, thereby reducing the existing wide yield gap in the farmers' fields (Ali and Gupta 2012). Amin et al.

(2015) and Asnake and Bejiga (2003) concluded that low-yielding varieties and the narrow genetic base are the major constraints in lentil production.

Over the centuries, traditional breeding practices have led to improvement of nutritional composition, color, shape and taste in lentil. Lentils have evolved into a wide range of cultivars, adapted to diverse agro-ecological zones and multicultural preferences. Traditional breeding has been successful in addressing major production constraints in lentil by the development of cultivars resistant or tolerant to major biotic or abiotic stresses (Materne and McNeil 2007; Muehlbauer et al. 2006; Sarker and Erskine 2006); however, lentil breeding through classical means is limited by the low genetic variability, lack of genetic information and accurate selection methods which restrict the breeders to pursue other achievable breeding goals. Due to the low yield and narrow genetic base of available lentil genotypes, plant breeding programs face shortage of genetic raw material for selection.

9.6 Germplasms Biodiversity and Conservation

Formal world germplasm collections of lentils are maintained for breeding purposes at ICARDA (http://www.icarda.org), the Indian Agricultural Research Institute (http://www.iari.res.in), the Vavilov Research Institute of Plant Industry (http://www.vir.nw.ru) and the USDA collection at the Regional Plant Introduction Station, Pullman, Washington (https://www.ars.usda.gov). Each institution has more than one thousand conserved accessions. In addition, there are other germplasm collections available for breeding and research purposes. The Global Crop Diversity Trust (https://www.croptrust.org/) records the global status of *Lens* ex situ genetic resources in major collections to devise new conservation strategies. The published reports of the trust show a large number of *Lens* accessions comprising wild relatives, landraces and advanced breeding materials available in collections worldwide. Worldwide, the leading 41 collections together hold 43,214 accessions of genus *Lens*. The major genebanks/institutes with more than 1000 *Lens* accessions are listed in Table 9.4. ICARDA, with 24% of the global *Lens* germplasm accessions, includes 583 wild accessions and is the largest such collection in the world.

In West Asia and North Africa, as centers of origin primary diversity, lentil is a high priority crop in regional conservation strategy, with first priority assigned to the crop in West Asia, and very high priority in North Africa. South, Southeast and East Asia conservation strategy gives lentil an overall priority of 17th out of 28 crops. The priority ranking for lentils in the South Asian subregion increases to 14th most important crop. Bangladesh, India and Nepal assign lentils to the highest priority category while it is at second priority in Bhutan and Sri Lanka. Lentil is the most preferred pulse crop in the subregion with rich genetic diversity in India and Nepal. A total of 3022 lentil accessions are conserved in the South Asian subregion; the largest collection at NBPGR, India and the working collection at IIPR, India.

Assessment of genetic diversity within South Asian lentil germplasm, using RAPD markers, revealed the lowest narrow diversity in Pakistan, Afghanistan and

Table 9.4 Major *Lens* spp. collections (>1000 accessions) in the world

Sl. No.	Country	Genebanks / institutes	Number of accessions
01	Global	ICARDA	10,822
02	Australia	Australian Temperate Field Crops Collection	5254
03	Iran	Seed and Plant Improvement Institute	3000
04	USA	USDA	2875
05	Russian Federation	N.I. Vavilov All-Russian Scientific Research	2556
06	India	National Bureau of Plant Genetic Resources	2285
07	Chile	Inst de Inv. Agropecuaria, Centro Regional deInvestigación Carillanca	1345
08	Canada	PGRC	1139
09	Turkey	Plant Genetic Resources Dept. Aegean Agricultural Research Inst.	1095
10	Syria	General Commission for Scientific Agricultural Research	1072
11	Hungary	Research Centre for Agrobotany	1061

Source: https://www.croptrust.org/

Nepal (Ferguson et al. 1998). Lentil breeders desire a wide selection of opportunities to initiate breeding, therefore, broadening of the genetic base and subsequent characterization and preservation is important for efficient utilization of germplasm in future lentil breeding activities. Induced mutagenesis has been done in lentils to improve the genetic base and to produce useful new mutants. Toker et al. (2007) reviewed the use of chemical mutagens and ionizing radiation to create new lentil mutants for commercial production. The FAO/IAEA mutant variety database (MVD) records mutant varieties released for cultivation around the world. Sarker and Erskine (2006) reported that there have been significant breeding achievements in lentil since the late 1970s. The genetic base of lentil has been broadened by incorporating target traits from wild *Lens* species, landraces and mutants and new improved breeding lines having tolerance to abiotic (drought and cold) stresses and resistance to biotic stresses. Because of the importance of *Lens* biodiversity, AGILE: Application of Genomic Innovation in the Lentil Economy, an on-going collaborative project, was initiated to characterize the genetic variability within the primary and secondary gene pools of *Lens* using extensive genotyping and phenotyping techniques for application into the lentil breeding programs (http://knowpulse.usask.ca/poratl/project).

In Asia, the ECHO Asia Seed Bank (https://www.echocommunity.org) maintains a unique collection of hard-to-find seeds of mostly underutilized crops including legumes that thrive under difficult growing conditions to serve development workers and community organizations working within Asia to improve the rural livelihood. In India, the M. S. Swaminathan Research Foundation (www.mssrf.org) is involved in the development of community based seed-grain-gene banks to keep the farming community engaged in promotion of sustainable agricultural practices. A

number of non-profit organizations in different parts of the world are maintaining seed/gene banks in close collaboration with farming communities to conserve plant genetic resources and thus provide a platform to initiate participatory plant breeding experiments.

Globally, a number of genebanks with large lentil collections are conserving diverse accessions of lentil genetic resources available for the purpose of breeding and research in future. However, experts on efficient and effective ex situ conservation of lentil and its wild relatives have reported gaps in the coverage of global genetic diversity in existing collections and also raised concern about the deteriorating conditions of some of the collections. The Global Seed Vault, also known as the Doomsday Vault, in Svalbard, Norway, has been in operation since 2008 to preserve a wide variety of plant seeds including lentil seeds collected from operational gene banks worldwide. The seed vault operates under an agreement between the Norwegian Ministry for Agriculture and Food, the Global Crop Diversity Trust and the Nordic Genetic Resource Center (NordGen), with Primary Funding from (https://www.seedvault.no). As of the end of 2016,a total of 918,910seed accessions were in the vault from international (600,566), national(284,130) and regional(22,583) gene banks, the university sector(7767), NGOs(3857) and private companies(7)(SGSV 2017).

9.7 Breeding Objectives

Lentil breeding objectives usually differ depending on the problems and priorities of farmers and consumers of a specific region of the world. Muehlbauer et al. (1995) pointed out that the main breeding goals in the key exporting countries are higher and stable seed yield, disease resistance and better seed quality; while increased yield per hectare is the primary aim in the import-dependent countries like India. In general, lentil breeding with genetically diverse parents having traits of interest is carried out to generate new gene combinations to increase yield and stress resistance (Sarker et al. 2009).

Lens culinaris ssp. *orientalis,* a miniature cultivated lentil which bears pods that burst open immediately after maturation, is considered the wild progenitor of lentil. Early farmers around 700 BC began selecting plants with more intact mature pods. Muehlbauer (1992) mentioned pure line selection within heterogeneous landraces as the most common method of cultivar development during the early phases of lentil improvement. These early selection efforts were successful in achieving species with decreased pod dehiscence and dormant seeds, while having a considerable increase in erect plant habits, seed size and color diversity (Zohary 1996). The application of pure line selection has been an important means to develop genetically uniform and locally adaptable lentil cultivars from local landraces or introduced germplasm. However, hybridization and selection have become the chief means of lentil improvement during the past three to four decades, especially in

developed countries. With increased global demand, crossbreeding is preferred for quick development of new lentil cultivars/genotypes.

The mode of reproduction is the key factor that determines plant genetic structure and thereby the crop breeding methods to be implemented. Lentil is predominantly a self-pollinating species with a very low rate of outcrossing; therefore, the methods of breeding lentil, and other self-pollinated crops, are similar and include pure line selection or hybridization followed by the combinations using the bulk method, the pedigree method, the single seed descent, or a modification of these procedures supplemented with mutation breeding (Muehlbauer et al. 2009; Toker et al. 2007) for specific purposes.

The availability of sufficient variability for a trait within the crop gene pool is necessary for the genetic manipulation of that trait through plant breeding for overall improvement of the crop. The lentil gene pool has adequate variability for many key traits; however, plant breeding efforts to address several other important traits are restricted by the low availability of genetic variations for those traits within cultivated germplasm and crossable wild species. Scarcity in genetic variability represents a limitation for lentil breeding.

9.8 Conventional Breeding Methods

The major goal of lentil breeding is the creation of varieties with higher and more stable yields, by exploiting genetic variability followed by the selection and evaluation of selected lines. Unlike other legume crops, lentil productivity is low with average yields of 570–766 kg/ha in Asia and 600–660 kg/ha in Africa. The successful outcome of conventional breeding depends on the degree of existing and accessible genetic variability. The main conventional breeding methods are discussed below.

9.8.1 Plant Introduction

Plant introduction is a method of obtaining high yield and wide adaptability of lentil cultivars from within or outside the country. The success of plant introduction depends on the introduced cultivar, as well as the soil and climatic conditions of the new location. Homozygous pure lines are suitable plant material in plant introduction due to their better adaptability, compared to a heterozygous segregating population, which requires identification of a productive line with specific desirable traits. Even though plant introduction is employed at the initial phase of a breeding program, it is a discontinuous process but the cheapest and fastest way of developing cultivars. Hence, it becomes more important in a country where there is less area under crop cultivation, economic restraints or the absence of trained staff. Introduction is generally facilitated in the following ways: (1) Exchange of cultivars

with other relevant plant breeders; (2) Area exploration showing higher species richness and evenness; (3) Obtaining plant genetic resources from national and international organization such as FAO, ICRISAT, ICARDA, NBPGR, India and the USDA plant introduction stations.

The introduction of lentils into the South Asian region took place in about 2000 BC from West Asia via Afghanistan (Cubero 1981; Materne and Siddique 2009). At present, ICARDA is leading in the lentil introduction program around the world, including South Asian countries, through the procedure of selection-introduction-acclimatization for popular cultivars from one country/region into another country with similar agroecological environments. As South Asia contributes around one-half the world lentil production, past attempts had been made to widen the narrow genetic base of endemic the small-seeded Pilosae type by introducing the bold-seeded Precoz type, of Argentine origin, from ICARDA in the lentil breeding programs in the region (Erskine et al. 1998). Lentil cultivars Vipasha and VL 507 in India; Mansehra 89 and Shiraz 96 in Pakistan; and Simal, Sikhar, Khajura Masuro 1 and Khajura Masuro 2 in Nepal were introduced from ICARDA (Rahman et al. 2009). Enrichment of local gene pools through a number of other introductions from different sources has also been carried out, which led to the development of improved cultivars and yield stability in the South Asian countries, including India.

9.8.2 Hybridization

Hybridization is the process of combining desirable traits from two or more parents into a single cultivar. The main objective of hybridization is to increase the degree of genetic variation.

9.8.2.1 Selection of Parents

The selection of appropriate parents plays a crucial role in the success of hybridization. Depending on the objective, the choice of parents is made as follows: only one parent is selected if the objective is to create a variety with higher yield and wider adaptability. The second parent must be chosen so that it complements the first parent. Diverse parents are selected, if creation of variation for the desired traits or broadening of the genetic base is the objective. To analyze the diversity and the combining ability of genotypes involved in hybridization, biometrical approaches are employed to ensure a rational and scientific basis in selection of the parents. Knowledge of genetic relationships between genotypes for parent selection in crossing programs is extremely important to achieve effective genetic gain through plant breeding programs. In addition to morpho-physiological markers that provide initial assistance in germplasm characterization, DNA-based markers facilitate precise

estimation of available genetic diversity. Recent lentil diversity assessments, within a global collection of cultivars and landraces using SNP markers, suggests that selection of divergent parental genotypes for lentil breeding programs should be based on genetic distance between genotypes, rather than geographical distance alone (Lombardi et al. 2014).

9.8.2.2 Crossbreeding Techniques

Crossbreeding in lentil is a tiresome job and the success of the artificial hybridization has a range of 20–50%, depending upon the genotypes involved, and climatic condition such as temperature and humidity. Since lentil flowers are tiny and fragile, emasculation and pollination usually cause injury to the floral parts, thus reducing the success rate. To increase the success rate of hybridization the following actions are recommended:

(a) Selection of appropriate size flower buds;
(b) Selection of lateral buds rather than the terminal ones (Sindhu et al. 1981);
(c) During emasculation and pollination, proper care is required to avoid any mechanical injury to the floral parts;
(d) Timing of pollination and fertilization is also vital in determining the success rate. Low temperatures, afternoon emasculation followed by pollination the following morning gives better results. However, high temperatures, morning emasculation followed by immediate pollination are also reported to be successful (Bejiga and Tessema 1981; Khosh-Khui and Niknejad 1972; Pundir and Reddy 1984).

Early efforts to introgress exotic genes into locally-adapted cultivars through hybridization resulted in selection of genotypes with key traits of interest (Rahman et al. 2009). Ladizinsky (1992) defined crossability as the potential of creating fertile F_1 hybrid production from intercrossing between individuals belonging to different taxa. Interspecific hybridization within the genus *Lens* shows crossability barriers within the species as well as between species (Ferguson et al. 2000; Ladizinsky 1997; Ladizinsky and Abbo 1993). The high crossability potential for accessions of *L. culinaris* ssp. *orientalis* and *L. culinaris* ssp. *culinaris* suggests that ssp. *orientalis* is the wild progenitor of the cultigen (Ladizinsky et al. 1984). Davey et al. (2005) suggested that interspecific hybridization barriers can be overcome through somatic hybridization using protoplast fusion techniques. However, successful reports on application of somatic hybridization and somaclonal variation techniques in lentil are very limited to date.

9.8.3 Mutation Breeding

The use of traditional breeding methods for a long period of time may have narrowed genetic variability. Further improvement in a crop species requires increasing novel genetic variability. From this point of view, mutations may be fruitful in plant-breeding programs. Induced mutation techniques, as a complementary breeding strategy, is a quick and cost-effective method to broaden the genetic base in lentil. The view that mutation breeding significantly helps in the augmentation and recreation of genetic variability for the development of many crop varieties is shared by many eminent scientists in the field (Branch 2002; Canci et al. 2004; Jain 2002; Jankowicz-Cieslak et al. 2017; Khan 1990; Maluszynski et al. 1995; Mba 2013; Micke 1988; Nakagawa et al. 2011; Oladosu et al. 2016; Riaz and Gul 2015; Shu et al. 2012; Toker et al. 2007; Tomlekova et al. 2014). However, the success of mutation induction for genetic improvement of crop plants depends directly upon the efficacy and nature of the mutations induced and the accuracy in their screening (Khan and Siddiqui 1992a,b; Manju and Gopimony 2009; Siddiqui and Yousufzai 1988).

9.8.3.1 Brief Historical Perspective

The term *mutation* was coined by De Vries (1901), to denote a sudden heritable change in the genotype of an organism. This may involve a change in its chromosome number, gross changes in the structure of chromosomes and changes in the individual gene; the process is known as *mutagenesis*. In a broad sense, except for recombination, mutation includes all types of heritable genetic change of an organism. Mutation provides the raw material for evolution; it is the ultimate source of all genetic variation and results in the evolution of alleles.

van Harten (1998) reviewed the history of mutation breeding, beginning with the earliest account of natural or spontaneous mutants in cereals presented in an ancient document entitled *Lulan* that appeared around 300 BC in China, up to the international coordinationinitiativefrom1964 onwards by FAO and the IAEA (FAO/IAEA). Maluszynski et al. (2004) considered the experiments by Stadler (1928) to induce genetic variation in plants using radiation as the initiation of mutation breeding. A mutant cultivar, the so-called *chlorine-type* in *Nicotiana tabacum* L., was the first reported commercially-induced mutation product.

A mutation is an irreversible alteration in the DNA sequence of a gene. Mutation in a gene can change the amino acid sequences of the protein encoded by the gene. There are two types of mutation:

(a) Spontaneous or Natural Mutation. Mutation can occur in natural population without any human involvement; these types of mutations are called *spontaneous mutation*. They may arise due to errors in DNA replication or spontaneous lesions and transposition of transposable elements during the normal growth of cells, or it may be due to the presence of various physical and chemical muta-

genic agents like agricultural chemicals, industrial wastes, etc. in the environment. Spontaneous mutations are very rare and recessive, and most cannot be used for plant breeding purposes.

(b) Induced Mutation. Mutations are induced through exposure to different physical or chemical agents such as gamma rays, EMS, DES, MMS, 6-Amino purine etc., which are useful in crop improvement. Mutagens act by causing a structural change in the DNA molecules as they react with the parental DNA that affects the base pairing capability of the altered nucleotide.

The most important objective of induced mutagenesis technique for crop improvement is to achieve a desirable frequency and spectrum of mutation. The expression of traits is a complex process involving many interdependent genes and the response of each gene varies with the type of mutagen while the induction of mutation in a target genotype is governed by the type of mutagen used, especially the selected concentrations. Therefore, proper selection of mutagens and their doses/concentrations is the key to unlock the vast array of possibilities in mutation breeding. To ensure the selection of appropriate concentrations of mutagens, analysis of bio-physiological damages and cyto-morphological effects are the most dependable indices that provide an estimation of effectiveness and efficiency of mutagen concentrations towards the target plant genotype. Both physical and chemical mutagens are widely used to induce mutations in plants. Srinivasachar and Mohandas (1971) stated that the efficiency of physical or chemical mutagen can be modified by combining it with another of its own kind or of a different kind, as a pre-post or simultaneous treatment.

9.8.3.2 Mutagens

A form of radiation or a chemical agent that induces a mutation of the genetic makeup of an organism is termed as *mutagen*. The mode of action and strength is mutagen-specific, and depends on target and use. In addition, the cost, accessibility, safety and suitable tissue are the other important factors that define the specificity of different mutagens and their utilization. The mode of action of mutagens may be direct by damaging the genetic material or a disruption of the normal cell cycle and replication mechanisms. Also, there is a class of promutagens that act through cellular processes to form mutagenic metabolites without being directly involved in mutagenic activity. Mutagens may be of physical (Table 9.5) or chemical (Table 9.6) type:

(a) Physical Mutagens: X-rays and gamma rays are non-particulate electromagnetic radiations with a wavelength of 10^{-11} to 10^{-7} cm. The physical properties and biological effects of both are similar, but they differ in origin. They are highly penetrating and sparsely ionizing. α-rays, β-rays and neutrons are particulate ionizing radiation. UV radiations are nonionizing radiation with low penetration capacity generally induce dimer formation and deamination of DNA bases.

Table 9.5 Examples of common physical mutagens

Mutagen	Characteristics
X-rays	Electromagnetic radiation penetrates tissue from a few millimeters to many centimeters, produced by X-ray tubes
Gamma rays	Electromagnetic radiation emitted by radioisotopes and nuclear reactors, very penetrating into tissue, sources are Co^{60} and Ce^{137}
Neutrons	A variety exist (fast, slow, thermal); produced in nuclear reactors, unchargedparticles penetrate tissue to many centimeters, source is U^{235}
Beta particles	Produced in particle accelerators or from radioisotopes, are electrons, ionize, shallowly penetrating, sources include P^{32} and C^{14}
Alpha particles	Derived from radioisotopes, a helium nucleus capable of heavy ionization, very shallowly penetrating
Protons	Produced in nuclear reactors and accelerators, derived from hydrogen nucleus, penetrate tissue up to several centimeters

Table 9.6 Examples of common chemical mutagens

Mutagen group	Examples	Mode of action
Base analogues	5-bromouracil, 5-romodeoxyuridine	Deletion, addition, frame shift
Related compounds	Maleic hydrazide, 8-Ethoxy caffeine	Chromosome breaking
Antibiotics	Actinomycin D, Mitomycin C, Streptonigrin	Chromosome breaking
Alkylating agents:		Alkylate various sites in DNA
Sulfur mustards	Ethyl-2-chloroethyl sulfide	
Nitrogen mustards	2-chloroethyl-dimethyl amine	
Epoxides	Ethylene oxide	
Ethyleneimines	Ethyleneimine	
Sulfonates	Ethyl methane sulfonate (EMS),Diethylsulfonate (DES)	
Diazoalanes	Diazomethane	
Nitroso compounds	N-ethyl-N-nitroso urea	
Azide	Sodium azide	Gene mutation
Hydroxylamine	Hydroxylamine	Base pair transition
Nitrous acid	Nitrous acid	AT ↔ GC; GC ↔ AT
Acridines dyes	Acriflavin, Proflavin	AT ↔ GC
Intercalating agents	Ethidium bromide,Proflavine daunorubicin	Block transcription and replication

(b) Chemical Mutagens: Chemical mutagens are easy to use, there is no requirement for specialized equipment, and they can induce mutations at a very high frequency. Compared to radiological methods, chemical mutagens tend to cause single base-pair (bp) changes, or single-nucleotide polymorphisms (SNPs) as they are more commonly referred to, rather than deletions and translocations

(Sikora et al. 2011). Chemical mutagens have been successfully employed to create genetic variations in mutation breeding programs to develop cultivars with improved yield and yield attributing traits (Maluszynski 2001).

In practice, nowadays, ionizing radiations are the most often used category of physical mutagens, including X-rays and gamma rays. Alkylating agents are the most potent category of chemical mutagens, which includes EMS and MMS. Among the different radiation agents, gamma rays are most preferable due to their shorter wavelength and higher energy level. Gamma rays are generated from radioactive decay of elements such as 14C (Carbon-14), 60Co (Cobalt-60) and radium. van Harten (1998) reported that gamma rays can cause considerable damage when they pass through tissue due to their great penetrating power. High-energy gamma rays, during the process of tissue penetration, produce positively-charged free radicals or ions from the collision of electrons with atoms of different molecules (van Harten 1998) and start a chain reaction where these ions collide with other molecules to cause the release of further electrons. Overall, a *core* of ions is formed along the path of each high-energy ray while it passes through matter or living tissues.

Chemical mutagens have been successfully employed in mutation breeding programs to create fresh genetic variations in different crop plants (Bhat and Wani 2017; Laskar et al. 2015a, b; Maluszynski 2001; Mohan and Mathur 2015; Talame et al. 2008). Physical and chemical mutagens are being used in genetic improvement programs on different plant species including pulses (Khan et al. 2006; Laskar and Khan 2014; Reddy and Annadurai 1992; Verma et al. 1999). Over the years, induced mutagenesis has been advocated as a valuable tool for plant breeding to overcome the genetic bottleneck of cultivated germplasm and create noble allelic combinations for agro-economic trait(s) which facilitate the selection of improved cultivars through mutation breeding(Amin et al. 2015; Branch 2002; Canci et al. 2004; Kumar et al. 2010; Laskar and Khan 2017; Maluszynski et al. 1995; Micke 1999; Nakagawa et al. 2011; Ranalli 2012; Toker 2009; Tomlekova 2010; Tomlekova et al. 2009; Wani et al. 2014; Xiong et al. 2016).

9.8.3.3 Induced Mutation Applications in Crop Improvement

Gustafsson (1947) pioneered the usefulness of mutations in genetic improvement of crop plants. Micke (1995) reported that sustained efforts of several researchers over the years eventually converted the randomness of induced mutation into a targeted event for specific economic benefits. Thereafter, researchers applied induced mutations for improvement of crop genetic resources in several crop plants worldwide (Amin et al. 2015; Brock 1965; Chhun et al. 2003; Gaul 1965; Goyal and Khan 2010a; Ilbas et al. 2005; Kharkwal 1996; Kozgar et al. 2014; Laskar and Khan 2017, 2018a, b; Mondal et al. 2017; Nakagawa et al. 2011; Oladosu et al. 2016; Rajput et al. 2001; Talame et al. 2008; Toker 2009; Wani and Khan 2006).

Mutations are inherited changes in the genetic material creating new genetic variation that allows organisms to evolve on the basis of their origin. Although the

concept of mutation was first developed by De Vries (1901), its practical significance was not demonstrated until later by Muller (1927) in *Drosophila,* Stadler (1928) in barley and maize and Goodspeed (1929) in *Datura* and *Nicotiana.* Because the time required to breed improved varieties is relatively short and it does not alter the whole genome, only a small portion is altered, these discoveries initiated the crop improvement programs through induced mutation. Later, induced mutation technique became a highly convenient tool for plant breeders to generate the much-needed genetic variation in different crop species, thereby providing them with the access to unexplored allelic combinations within the crop genome for crop improvement programs around the world. Several countries including India, China, USA and Japan have adopted mutation breeding as a tool for crop improvement. By the end of twentieth century, more than 3000 mutant varieties of crop plants, including cereals, oilseeds, pulses, vegetables, fruits, fibers and ornamentals had been developed, as recorded in the IAEA/FAO mutant variety database.

The availability of genetic variation in the traits of interest is a prerequisite for any crop improvement program. Mutation breeding offers an unpredictable possibility of inducing desired attributes in the crop that are either novel in nature or have been lost amid development (Novak and Brunner 1992). Micke (1999) advocated the mutation approach as much better than other methods due to the rapid generation of sufficient mutations. Mutation breeding supplements conventional crop improvement in a favorable manner by making it possible to upgrade a specific character without altering the original genetic make-up of the cultivar (Gottschalk 1986; Toker et al. 2007). It accelerates the crop improvement and shortens the time required for variation generation at pre-breeding stages, unlike the extensive hybridization and backcrossing methods used in conventional breeding. The other option of transposon or T-DNA insertional mutagenesis for generating genetic variations usually results in a complete disruption of gene function; moreover, the nonrandom distribution of insertion sites requires a greater number of insertion lines to cover the full genome, and is thereby unable to provide the desirable range of mutation for crop improvement (Chopra 2005; Parry et al. 2009; Zhang et al. 2007). Conversely, chemical and physical mutagens introduce random mutations in all target genes throughout the genome; therefore, an individual plant genome can exhibit a wide range of mutations, which allows for small population sizes for better management during crop improvement (Parry et al. 2009).

Mutation breeding techniques are believed to be the best approach for broadening the genetic base and overcoming the genetic bottleneck of lentils (Erskine et al. 1998; Toker et al. 2007), particularly the Indian lentils. Gaul (1964) classified mutations phenotypically into two forms:

(a) Macromutations: Easily detectable changes in individual plants, phenotypically visible and morphologically distinct, and qualitatively inherited genetic changes, occur in major genes or mono/oligogenic traits.
(b) Micromutations: Result in a small effect that, in general, can be detected only with the help of statistical methods and quantitatively inherited genetic changes, and occur in minor genes or polygenic traits.

Most agro-economic traits like yield are polygenic in nature, thus the proposed hypothesis by Brock (1965) of induction of quantitative variability through mutagenic treatments has heightened the interest of plant breeders to widely generate micromutations. Micromutations require careful observational strategies for accurate selection of quantitatively-inherited traits like yield and to isolate the best mutants with intact basic phenotypic architecture. There are sustained efforts worldwide to assess mutagen-induced genetic variability in quantitative traits in pulse crops However, lentil, being a self-pollinated, annual diploid with a relatively unexplored narrow genetic base, needs substantial research to increase productivity and reduce the yield gap. Lentil mutation breeding requires careful selection of mutants with altered morphological architecture (Fig. 9.3) relevant to the yield and yield-attributing traits.

Mutation induces changes at loci that control important traits and/or by eliminating undesirable genes from elite breeding lines. Mutation breeding makes it possi-

Fig. 9.3 Mature lentil mutagenized plants that are in the process of in-field selection for determination of yield and harvest index

ble to improve only one or two traits without affecting the rest of the genotype (Shu et al. 2012). Many economically-important cultivars have been produced through mutation breeding (Ahloowalia et al. 2004) and in genetic improvement of crops; induced mutations represent a complimentary approach (Mahandjiev et al. 2001). Mutations have led to cultivars with greater yield and good seed quality, thus improving agronomic inputs and consumer acceptance (Ahloowalia et al. 2004). Initially mutation breeding was based primarily upon X-rays, gamma rays, thermal neutrons and radio isotopes of certain heavy elements; however, the discovery of chemical mutagens is considered to be a milestone in the history of induced mutations.

The reports of two chemicals, iodide and copper sulfate, known to act as weak mutagens on *Drosophila*, in the 1930s and the use of chemical mutagen ofurethane during World War II (Donini and Sonnino 1998), were the initial demonstration of chemical mutagen activity. Within a decade after discovery of the phenomenon, the utility of chemical substances as potential agents of mutation started establishing itself with reports from different locations. Auerbach and Robson (1942) were the first to present an elaborate report on induced mutations, as well as chromosomal breaks, by mustard gas in *Drosophila*. A number of chemical mutagens are now known to induce mutations in plants when applied singly or in combination, successively or simultaneously, with physical mutagens (Ahloowalia and Maluszynski 2001; Encheva 2009; Goyal and Khan 2010b; Khan et al. 2011; Konzak et al. 1965; Saleem et al. 2005). Sharma (1985), van Harten (1998), Micke (1999) and Kodym and Afza (2003) contributed significantly to our present knowledge of fundamental mutational processes and possible modes of action of various mutagenic agents. Several other researchers have also reviewed the properties and action of physical and chemical mutagens in different plant species that considerably exaggerated the usefulness of induced mutation in crop improvement (Ahloowalia et al. 2004; Al-Qurainy and Khan 2009; Amin et al. 2015; Gottschalk 1978a, b; Gottschalk and Wolff 1983; Goyal and Khan 2010a; Kaul 1989; Kaul and Nirmala 1999; Khan 1997; Laskar et al. 2018a; Nakagawa et al. 2011; Siddiqui 1999).

Gamma rays are ionizing radiation and have excellent penetration capability, with an energy level from around 10 kilo electron volts (keV) to several hundred keV. Gamma radiation absorption and its effects on biological material are deeply influenced by plant characteristics and degree of irradiation. Radiation predominantly targets the DNA for the biologic effects (Han and Yu 2010). The action mechanism of radiation on cellular DNA can be direct or indirect, in which DNA is hit by the radiation directly, thereby disrupting the molecular/genome structure, or the radiation hits the water and other organic molecules present in the cell to produce free radicals (Desouky et al. 2015). Saha (2013) reported that the majority of radiation-induced damage results from the indirect mode of action because water constitutes nearly 70% of the cell.

Induced reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide react to damage or modify almost all important structural and functional organic molecules, including proteins, lipids and nucleic acids, and are reported to differentially affect the morphology, anatomy, biochemis-

try and physiology of plants (Kebeish et al. 2015). Additionally, induction of reactive nitrogen species (RNS) and other species may involve cellular damage (Wardman 2009), and also the ionization of atoms on DNA molecules. In the case of either direct or indirect effects, the manifestation of the ultimate results originate from the biological and physiological alterations, which may be perceptible within seconds or not for decades (Desouky et al. 2015). Gamma rays have been the most successful mutagenic agent to induce a broad spectrum of mutations in large numbers of crop species (Goyal et al. 2009; Javed et al. 2000; Laskar et al. 2018b; Momin et al. 2012; Raina et al. 2017).

The application of induced mutagenesis on higher plants have revealed that chemical mutagens, owing to their relatively low cost, easy handling, mild effect and greater specificity, are preferable to physical mutagens which cause chromosomes to break (Auerbach 1965; Handro 1981; Laskar et al. 2015a, b; Salnikova 1995). An overwhelming majority of the strong chemical mutagens were discovered by Rapoport (1966), and have been used extensively in genetic studies and breeding research. Chemical mutagens can be classified into three groups: alkylating agents like ethyl methanesulphonate (EMS), methylmethanesulphonate (MMS), ethylethanesulphonate (EES), ethylene imines, diethylsulphate (dES), nitroso compounds; base analogues like 5-bromo uracil, 5-bromo deoxyuridine; and others like azides (sodium azide), antibiotics, acridines, nitrous acid and hydroxylamine. Half-life, the length of time required for the active reaction agent of a chemical mutagen to fall to one-half its initial value, is the measure of the rate of hydrolysis of the chemical mutagens. Alkylating agents are the most used group of chemical mutagens, which can be broadly classified as monofunctional and bi- or polyfunctional, depending upon the number of alkyl groups present in the compound (Natarajan 2005). Because they are more effective than physical mutagens, chemical mutagens have become the preferred method of induction of mutation, even with the advent of more modern technologies (Blixt and Mossberg 1967; Greene et al. 2003; Jain 2002; Kharkwal 1998; Perry et al. 2003). Several researchers confirm that chemical mutagenesis is most effective and efficient in lentil (Gaikwad and Kothekar 2004; Sarker and Sharma 1989; Solanki and Sharma 1994, 1999; Toker et al. 2007) and in other pulses (Bhosle and Kothekar 2010; Kharkwal 1998; Shah et al. 2008; Waghmare and Mehra 2000).

Srinivasachar and Mohandas (1971) reported that the efficiency of physical or chemical mutagen can be modified by combining either with another of its own kind or of a different kind as a pre-post or simultaneous treatment. Mehandjiev (2005) advocated the possibility of induction of a wide spectrum of mutation by the combined treatments of physical and chemical mutagens. van Harten (1998) suggested that combined treatments would create a synergistic effect that can enhance the mutation frequency or induce noble mutations, compared to individual mutagen treatment. Various reports support the usefulness of combination treatments over individual physical or chemical mutagen treatments for the improvement of lentil and other pulses like soybean *Gylcine max* (L.) Merr. (Khan and Tyagi 2010; Patil et al. 2004); black gram *Vigna mungo* (L.) Hepper (Goyal and Khan 2010b; Usharani and Kumar 2015); mung bean *Vigna radiate* (L.) Wilczek (Grover and Tejpaul 1982;

Singh 2007); fava bean *Vicia faba* L. (Bhat et al. 2007); cowpea *Vigna unguiculata* (L.) Walp. (Bind et al. 2016; Girija and Dhanavel 2009) and chickpea *Cicer arietinum* L. (Kamble and Patil 2014).

9.8.3.4 Past Achievements

The present era of changing climatic conditions is detrimental to crop production and food supply to provide for a rapidly increasing human population. Therefore, the existing need for sustainable agricultural intensification to meet the pressing food demand can effectively be achieved through development of new high-yielding crop varieties with wide adaptability. Lentil is an excellent dietary staple because of its high protein content and nutrient density. Therefore, generating polygenic variability for quantitative traits in lentils to achieve maximum economic use of the plant in the fight against food insecurity and malnutrition is highly recommended. In lentil, mutation breeding has contributed to the development of 18 mutant varieties worldwide. A listing of mutagenic lentil cultivars released globally is presented in Table 9.7. Among these, two cultivars, Ranjan and Rajendra Masoor 1, have been released from India for different improved traits like high yield and spreading type, tolerance to low temperatures, early maturity and suitable for late sowing. India contributed 55 mutant legume varieties to the tally of 287 from Asia and 453 total worldwide (Table 9.8). Of the total mutant varieties, only 15 (0.55 %) and 2 (0.06%) mutant varieties of lentil were released in the world and in India, respectively. It is also noteworthy that the two lentil mutants released in India were during the 1980s; no new varieties have been released in recent decades. Overall these statistics indicate the unexplored status of lentil genetic resources and the huge potential for mutation breeding, especially in high demand countries like India. Recently, a novel *multipodding* (mp) lentil mutant has been reported, that suggests a strong impact of inflorescence traits on lentil yield and yield stability (Laskar et al. 2018c). The widespread use of induced mutants in plant breeding programs throughout the world also suggests that the possibility of a larger number of mutants in cultivation than those documented in the FAO/IAEA database; efforts should be made to link the different country and institute based databases to the master FAO/IAEA database.

9.9 Biotechnology Applications

Lentil breeders have been successful in improving a number of monogenic traits using the selection-recombination-selection cycle of conventional plant breeding techniques. However, most of the economic traits like seed yield are quantitative in nature with a complex polygenic mode of inheritance. Therefore, execution of conventional plant breeding techniques for the improvement of genetically complex traits faces certain difficulties in precision, management and time durations. With

Table 9.7 Lentil varieties developed through direct induced mutation

Variety Name	Country	Year	Mutagen used	Main improved attributes
S-256 (Ranjan)	India	1981	X-rays irradiation	High yield and spreading type
Rajendra Masoor 1	India	1996	Gamma rays, 100Gy	Tolerance to low temperatures, early maturity and good for late sowing
NIAB Masoor-2006	Pakistan	2006	Gamma rays, 200Gy	Higher number of pods, resistance to lodging and resistance to blight and rust
Binamasur-1	Bangladesh	2001	extract of Datura seeds	High yield, tolerant to rust and blight, black seed coat
Binamasur-2	Bangladesh	2005	Gamma rays, 200Gy	High yield, early maturity, tolerant to rust and blight
Binamasur-3	Bangladesh	2005	EMS, 0.5%	High yield, early maturity, rust and blight tolerance
Mutant 17 MM	Bulgaria	1999	Gamma rays, 40 Gy	Vigorous growth habit, large leaflet, pods and seeds, resistance to anthracnose, stemohylium and viruses, high yield, drought tolerance and improved cooking quality
Zornitsa	Bulgaria	2000	EMS, 0.1%	High yield, high protein content (28.7%), good culinary and organoleptic quality, resistance to anthracnose, viruses and ascochyta blight
Djudje	Bulgaria	2000	Gamma rays, 30Gy	High yield, dwarf bushy habit, suitable for mechanized harvesting, non-shattering, resistance to fusarium and botrytis, high protein content (27.9%), good culinary and organoleptic quality
Elitsa	Bulgaria	2001	Gamma rays, 40Gy	High yield (34.4%) and resistance to the major disease
Verzuie	Moldova	2007	Gamma rays, 250Gy	Drought resistance, vegetative period (98 days), proteins26.7%, oils1.5%, fructose0.17%, glucose 0.08%, saharose1.23%, starch 45.30% and cellulose 7.16%
Aurie	Moldova,	2008	Gamma rays, 250 Gy	Drought resistance, high yields, early maturity, high protein content
NIAB Masoor-2002	Pakistan	2002	Recombinant	Erect growth habit, early maturity(120 days), black seed coat color, high grain yield, diseases resistance and synchronous pod maturity
Binamasur-6	Bangladesh	2011	Gamma rays, 250Gy	Higher yield (1.9 mt/ha and maximum yield 2.0 mt/ha), short maturityduration (105–110 days)
Binamasur-5	Bangladesh	2011	Gamma rays, 200Gy	Higher yield (2.1 mt/ha and maximum yield 2.2 mt/ha), short maturityduration (99–104 days)

(continued)

Table 9.7 (continued)

Variety Name	Country	Year	Mutagen used	Main improved attributes
Binamasur-8	Bangladesh	2014	Gamma rays, 200Gy	Higher yield (2.3 mt/ha and maximum yield 2.4 mt/ha),short maturityduration (95–100 days)
Binamasur-9	Bangladesh	2014	Gamma rays, 200Gy	Higher yield (2.1 mt/ha and Maximum yield 2.2 mt/ha),short maturityduration (99–104 days)
Binamasur-11	Bangladesh	2017	Gamma rays, 200Gy	Plants are erect, taller, many-branched and have green and violet colored flowers,higher yield (2.2 mt/ha and maximum yield 2.4 mt/ha),short duration (108–110 days)

Source: Joint FAO/IAEA, Vienna, Mutant Variety Database (MVD) 2018**;** http://mvgs.iaea.org

Table 9.8 Legume and lentil varieties developed through mutation breeding

	Mutant Varieties		
Crop	World	Asia	India
Lentil	18	12	02
Legume	453	287	55
Total	3281	1993	335

Source: Mutant Varieties Database, IAEA/FAO, 2018

the advent of modern biotechnological tools such as recombinant DNA technologies, molecular-marker based technologies and bioinformatics, lentil breeders are now able to introduce new genetic variability into the cultivated gene pool, identify the background genetic network and introgress desirable traits into cultivars using genomics-assisted breeding and genetic engineering techniques more precisely, within a short period of time.

9.9.1 In Vitro Culture

The application of in vitro culture techniques for management of genetic variability and acceleration of conventional breeding process is valuable in pulse crops breeding (Gatti et al. 2016). One of the essential necessities for success in genetic transformation using *in vitro* culture is a reliable regeneration protocol. Unfortunately, despite a number of reports on lentil regeneration, no suitable and reproducible protocol has been reported. In vitro culture of lentil is difficult due to its recalcitrant nature (Sarker et al. 2003; Gatti et al. 2016), compared to the success attained in other grain legumes. Micropropagation, consisting of somatic embryogenesis and organogenesis, by definition (Cruz-Cruz et al. 2013) is the in vitro vegetative propagation of plants to ensure rapid multiplication and production of plant material under aseptic conditions. These techniques offer easy and quick exploration of plant

Table 9.9 Some recent reports showing explant types used in somatic embryogenesis and organogenesis with success in regeneration of complete plants in *Lens culinaris* Medik

Mode of regeneration and explant type	References
Somatic embryogenesis	
Cotyledonary tissue	Chhabra et al. (2008)
Seeds	Chopra et al. (2011)
Organogenesis	
Decapitated embryos	Omran et al. (2008), Bagheri et al. (2012), Das et al. (2012)
Cotyledonary node explants	Sevimay et al. (2005), Chhabra et al. (2008), Bermejo et al. (2012), Özdemir and Türker (2014)
Cotyledons with a small part of the embryo axis	Tavallaie et al. (2011)
Shoot explants	Khentry et al. (2014)

genetic resources to breeders and there are successful reports in lentil with different explants (Table 9.9). In the last 30 years, techniques have progressively improved. Bajaj and Dhanju (1979) achieved the first partial success using meristem tips as explants and obtained *in vitro* lentil regenerants, while Polanco et al. (1988) reported multiple shoot formation from shoot tips. Williams and McHughen (1986) put forth a protocol for lentil regeneration from the hypocotyl and epicotyl-derived callus cells. Omran et al. (2008) reported a quick, effective and reproducible protocol for *in vitro* shoot regeneration by employing various explants and different concentrations of BAP (6-Benzylaminopurine, benzyl adenine). The results revealed successful *in vitro* shoot regeneration with a slight modification in the Murashige and Skoog (MS) medium presenting great promise. To a great extent, higher levels of BAP facilitated shoot regeneration in lentil genotypes. Additionally, decapitated embryos were the ideal explants for the highest shoot regeneration. Composition of salts and vitamins in medium has a prominent effect on *in vitro* culture of lentil. In other words, the B5 vitamins and increase of calcium concentration is necessary to overcome shoot-tip necrosis, a common problem in invitro culture of lentil genotypes. One of the major difficulties is the induction of functional roots on shoots developed through in vitro lentil micropropagation. MS medium supplemented with 25 mg/l indole butyric acid (IBA) showed 30% rooting efficiency (Sarker et al. 2003). The use of cytokinin in the initial explants for multiple shoots regeneration has been linked to problems of root induction at a later stage (Mohamed et al. 1992).

In vitro induction of flowering followed by pod formation and seed set directly from in vitro regenerated shoots was successfully demonstrated by Sarker et al. (2012) and Das et al. (2012) in lentil, which is remarkable for improving and shortening the breeding process. The wild genetic resources of lentil are valuable source of many novel gene(s) of agricultural interest and overcoming the fertilization barriers for hybridization between genepools are challenging in lentil improvement programs. In lentil, in vitro embryo rescue technique has been applied for introgression of anthracnose resistance from *Lens ervoides* (Fiala et al. 2009; Tullu et al. 2013) and *L. Lamottei* (Saha et al. 2015), ascochyta blight resistance from *L. ode-*

mensis, stemphylium blight, and resistance from *L. Tomentosus* (Saha et al. 2015) as well as genes useful to improve seed size (Tullu et al. 2013). In vitro cultures of immature lentils embryos/seeds have also been reported for further reducing the multiyear breeding cycles to 8 generations (Mobini et al. 2014).

Until now, *Agrobacterium tumefaciens* (Lurquin et al. 1998), biolistic transformation including electroporation (Chowrira et al. 1996) and particle bombardment (Gulati et al. 2002; Mahmoudian et al. 2002) have reportedly mediated gene transfer in lentil. The first transient and stable chimeric transgene expression on cotyledonary lentil nodes using particle bombardment was reported by Oktem et al. (1999). Transgenic lentils were produced successfully with *nptII* and *gus*A genes (Akcay et al. 2009) and *DREB1A* gene (Khatib et al. 2011). Subroto et al. (2012) developed transgenic lentil shoots using *A. tumefaciens* in two microsperma varieties. Transgenic lentil shoots were produced with an efficient of 1.009 %. Bermejo et al. (2012) reported an efficient and reproducible protocol for in vitro shoot regeneration from cotyledonary node explants and subsequently developed transgenic lentils with an overall frequency of 7 % (Bermejo et al. 2016). Atif et al. (2013) extensively reviewed transgenics production in range of different legume species. Although, these reports have established the possible techniques for transformation in lentil, improved transgenic lentil varieties are still to be developed and made available for cultivation.

9.9.2 Marker-Assisted Selection (MAS)

MAS provides a remarkable enhancement in the efficiency with which breeders can select plants with elite combinations of genes. A marker is a *genetic tag* that identifies a particular location within a plant's DNA sequences. Currently, the application of MAS in lentil-breeding programs is very limited due to inadequate availability and slow development of lentil genomic resources, as compared to other major legumes (Kumar et al. 2014). The morphological markers which were categorized as qualitative markers, because they exhibit monogenic dominant inheritance, include cotyledon (Yc),anthocyanin in the stem (Gs), pod indehiscence (Pi), seed coat pattern (Scp), flower color (W), radiation frost tolerance locus (Rf), early flowering (Sn) and ground color of the seed (Gc) (Duran et al. 2004; Eujayl et al. 1998; Hamwieh et al. 2005a, b; Tullu et al. 2003). Duran et al. (2004) quantitatively mapped inherited traits and detected five QTLs each for the height of the first ramification and flowering time, three for plant height, seven for pod dehiscence, and one each for shoot number and seed diameter. Five and four QTLs were identified that conferred winter survival and winter injury, respectively, using a RIL population of 106 lines derived from WA8649090 × Precoz (Kahraman et al. 2004). Saha et al. (2010a,b) identified a QTL imparting resistance to *Stemphylium* blight and rust diseases by using RIL populations. Quantitative traits are influenced by both genetic and environmental effects, hence, RILs or near-isogenic lines (NILs) are more appropriate populations to precisely dissect their components.

Zamir and Ladizinsky (1984) initiated linkage analysis in lentils and Havey and Muehlbauer (1989) developed the first DNA marker-based genetic map in lentil. Subsequently, with the development of PCR-based markers, several researchers have reported a number of lentil genetic maps. Eujayl et al. (1998) were first to publish an extensive linkage map of lentil comprising RAPD, AFLP, RFLP and morphological markers using an interspecific cross between *Lens* ssp. *culinaris* and ssp. *orientalis*. Rubeena and Taylor (2003) reported the first intraspecific lentil map comprising 114 RAPD, inter simple sequence repeats (ISSR) and resistance gene analog (RGA) markers molecular maps derived from intraspecific mapping populations are more convenient than those derived from intraspecific mapping populations for identification of desirable QTLs and gene tagging. Hamwieh et al. (2005a, b) constructed the first genomic library from lentil cultivar ILL5588. A set of 30 initially and later an additional set of 14 highly polymorphic well-distributed SSR markers were developed for genetic diversity analysis of lentil (Hamwieh et al. 2009). Verma et al. (2014) reported the development of 122 functional SSR markers for employment in lentil improvement programs.

Tanyolac et al. (2010) reported a molecular linkage map of lentil using AFLP, ISSR, RAPD markers and constructed 11 linkage groups covering 1396.3 cM with an average map distance between framework markers of 8.4 cM. To date, several genetic linkage maps have been constructed from lentil mapping populations, but low marker density and long length (in cM) of these linkage maps limits their practical applications (Ates et al. 2018a). Creation of consensus linkage maps using multiple mapping populations, instead of a single mapping population based conventional linkage maps, has gained major prominence in recent times due to significant advancements in biotechnological tools and molecular markers. Currently, DNA chip-based markers, particularly with SNPs, are gaining popularity over PCR-based markers, as potential markers for next generation sequencing (NGS) approaches. Sharpe et al. (2013) identified about 44,879 SNP markers in lentil using the Illumina Genome Analyzer. Another set of 50,960 SNPs were identified and used to construct a lentil linkage map (Temel et al. 2014). With progress in the lentil genome sequencing using next generation technologies (Bett et al. 2016), candidate genes for several agronomic and quality traits have been identified, which will enable breeders to implement marker-assisted selection in lentil breeding. A high-density consensus linkage map consisting of seven linkage groups, representing seven chromosomes of the lentil genome covering a total of 977.47 cM with an average distance of 0.10 cM between adjacent markers, has been constructed using Diversity Arrays Technology (DArT) (Ates et al. 2018a).

9.9.3 Next Generation Technologies

In the past, lentil's narrow genetic base and unavailability of genomic resources have thwarted the efforts of breeders to employ molecular techniques in mainstream breeding programs. Kumar et al. (2015) listed some of the major limiting factors

such as large genome size, narrow genetic base, lack of candidate genes and low density linkage map that restricts the development and application of genomics assisted breeding in lentil. The progress in next generation sequencing (NGS) technology and genotyping by sequencing technologies (GBS) has opened new opportunities for speedy development of sequence-based markers and accelerated the progress of lentil genome sequencing projects around the world. Bett et al. (2016) have released a lentil genome assembly based on a well-known small red lentil variety, CDC Redberry, using next generation DNA sequencing technologies. The reported lentil assembly consists of seven pseudomolecules, anchored through the use of six high-density genetic linkage maps, with the total assembled bases representing approximately half of the 4.3 Gb lentil genome (https://www.genomecanada.ca) and accessible through the KnowPulse web portal (http://knowpulse.usask.ca). A genomic map facilitates the identification of underlying gene(s)/QTL governing the variations in trait(s) of interest. Although there is a considerable increase in the number of available molecular markers for genetic mapping in *Lens*, a complete genetic map is still lacking in lentil. Therefore, a comprehensive consensus genetic linkage map of lentil needs to be developed by including both morphological and molecular markers simultaneously, to allow tagging of key biotic and abiotic stress resistance genes, for further exploitation in plant breeding with increased selection accuracy. Bett et al. (2016) also stressed the importance of conducting field phenotyping of available lentil germplasms in different lentil growing regions, including South Asia, for development of molecular markers for traits of interest.

Gupta et al. (2012) reported three QTLs for resistance to *Ascochyta* blight at seedling and pod/maturity stages. Recently, Khorramdelazad et al. (2018) revealed key defense response genes against *Ascochyta* blight, caused by the fungus *Ascochyta lentis*, using transcriptome profiling of lentil resistant ILL7537 and susceptible ILL6002 genotypes via a targeted RNA-Seq approach during host-pathogen interactions. Sari et al. (2018) also identified candidate defense genes differentially expressed among genotypes through RNA-seq analysis and quantitative real time-PCR (RT-qPCR),useful for designing molecular markers suitable for *Ascochyta* blight resistance gene pyramiding in lentil. Increasing the concentration of micronutrients in pulses could be the most promising approach to eradicate micronutrient malnutrition, as developing countries with large population are the worst affected, where lentils along with other pulses play a major role in human diet. In the last two decades, considerable progress has been made in biofortification of lentil cultivars through plant breeding; scientists now have access to modern molecular techniques and are better equipped to deal with the problem of prevailing malnourishment. Recent construction of high density linkage maps and identification of QTLs for Fe (Aldemir et al. 2017) and Mn (Ates et al. 2018b) concentrations in the lentil genome through next-generation sequencing technologies will enhance future plant breeding activities for biofortification studies in lentil.

The high frequency of genomic variations induced by physical and chemical mutagens is detectable with the single nucleotide polymorphisms (SNPs) screening method, thereby accurately identifying mutant traits at the molecular level. Information about these induced sequence variations can now be linked to traits

using reverse genetic techniques to investigate gene function. Target-induced local lesions in genomes (TILLING), a high-throughput technique to identify single nucleotide mutations in a specific region of a gene of interest, can contribute significantly to an understanding of the function of gene(s) underlying a trait of interest in mutagenic lentil populations. Likewise of importance is the application of other non-transgenic approaches, including RNAi technology and virus-induced gene silencing (VIGS), which contribute to an understanding of the molecular mechanisms of biological activities in lentil. All these innovations have led to an increase in genomic resource utilization in lentil genetic improvement and are accelerating development of improved cultivars. Simultaneously, these advances will also encourage lentil breeders in developing countries, where activities are still at a preliminary stage, to incorporate marker assisted selection (MAS) and marker-assisted backcrossing (MABC) in lentil breeding programs for selection and introgression of more unconventional traits to target yield.

Enhancing conventional breeding approaches with modern biotechnologies is helpful to better utilize the available genetic variability in cultivated germplasm. Supplementary breeding-induced mutations of unexploited traits can increase the genetic variability in cultivated lentils. With respect to lentil mutation breeding, the future impact of these new ideas and methodologies will be in the introgression or pyramiding of mutant alleles through marker-assisted backcrossing and enhanced outline of mutation breeding through marker-assisted genotypic selection for essential attributes. In future, induction of mutations in new gene(s) like inflorescence architecture gene(s) and development of reliable linked molecular markers would definitely accelerate the selection and introgression of novel and economically-important trait(s) like multipodding or a determinate growth habit into lentil breeding lines. More resources should be directed, especially in India, towards the identification of new markers, development of fresh linkage maps for identification of novel QTL/genes for yield, nutrition and adaptability traits in lentil.

9.10 Conclusions and Prospects

Lentil is preponderantly an autogamic crop with variable>1–6% allogamy. Thus breeding methods pertinent to each self- and cross-pollinated crop could be applied to lentil improvement. Plant breeders must set specific objectives before beginning breeding work. Therefore, in the case of lentils, initially the breeder must recognize the necessities of the farmers, the target environments together with the biotic and abiotic stresses, consumer preferences, and the on-form production practices and the food processing industry. In general, the objectives of lentil breeding ultimately revolve around yield and yield stability, disease and pest resistances, drought, salinity and high-temperature tolerances, enhancing grain protein quality, earliness, wider adaptability as well as other agronomic attributes. Also, the selection of a precise breeding strategy in lentils depends on the sort of cultivar (varieties/mutants/hybrids) intended to be created. Because high value seed yield is a polygenic trait

with a complex mode of inheritance, direct selection for yield is rarely effective in lentil breeding. To attain desirable yield potential, it is essential to identify new traits that can have a major impact on total yield and are also necessary to generate new information on the genetics of complex traits for molecular tagging of the responsible genes.

The importance of hereditary markers as alternate determination pointers in plant breeding has been noted for over 90 years. Nonetheless, it was not until the mid-1980s that well-endowed molecular markers became accessible for dependable determination of agronomically-imperative qualities in breeding programs. From that point forward, indirect selection utilizing DNA markers essentially extended the adequacy and pace of plant breeding. The twenty-first century also witnessed another major step forward with the arrival of machine-controlled technologies, cutting edge DNA sequencing and empowering statistical- and bioinformatics-based tools. The rapid advancement of DNA-marker procedures and genomics in recent decades is changing the way conventional and mutation-based plant breeding is being refined. For instance, procurement of nucleotide variations through sequencing permits the improvement of simple PCR-based markers for genotyping tests; for example, allele-specific amplification, high-resolution melt analysis (HRM), cleaved amplified polymorphic sequences (CAPS) and other assays of different measures. Molecular strategies for crop improvement are becoming progressively mechanized and reliable, and costs have declined considerably. Fundamentally for plant breeding, the accessible high throughput sample analysis methods permit the investigation of a large number of tests with high precision in a short time period. While numerous techniques have been portrayed, the current paradigm is that direct DNA sequencing is becoming the standard stage from which new methodologies are being launched. The utilization of sequencing in this way gives new and exact apparatuses, and associatively new systems, for both mutation recognition and marker-assisted selection. Implementation and utilization in recent years of clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) systems have spawned a new era in plant genetic engineering (Rojo et al. 2018).

The major steps in a plant breeding program are inducing genetic variability in the target trait(s), recognizing the desired variability for target trait(s), utilizing that variability, and advancing the desired recombinants in subsequent generations to develop final crop genotype(s) with desired gene(s) responsible for the target trait(s). Conventional breeding approaches are helpful to utilize the available genetic variability in the cultivated germplasm, which has resulted in the development of several high yielding and better adaptable varieties of lentil. In the last decade, several linkage maps have been developed and QTLs identified for the traits of interest in lentil. This has paved the way for mainstreaming genomics-enabled improvement in lentil breeding. Currently the regular use of markers for marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) in lentil breeding is very limited. It will get an additional boost once the draft genome sequence and resequencing of the reference set of lentil is completed. One of the major reasons for the slow

progress in the study of lentil genomics may be the constraints of developing economies and technological inaccessibility in high-lentil growing countries with ample genetic resources, as well as negligible importance given to lentils in the past as compared to the major pulse crops. However, population growth in developing countries and changing food habits in developed countries have increased the market demand and value of lentils. Lentil now is considered a target crop for research and development among the pulse crops.

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Appendices

Appendix I: Research Institutes relevant to Lentils

Institution	Specialization and research activities	Contact information and website
University of Saskatchewan, Canada	Teaching and research institute, pulse crop genomics, genetics of domestication and adaptation in lentil, lentil genome sequencing,molecular marker development	Prof. Kirstin E. Bett, College of Agriculture and Bioresources, Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada. https://agbio.usask.ca Email: k.bett@usask.ca
The University of Western Australia, Australia	Teaching and research institute, lentil breeding, germplasm evaluation and genetics, cultivar development	Prof. William Erskine, Director, Centre for Plant Genetics and Breeding, School of Agriculture and Environment, Faculty of Science, The University ofWestern Australia, Australia. http://www.pgb.plants.uwa.edu.au/ Email: william.erskine@uwa.edu.au
Washington State University, USA	Teaching and research institute, development of improved varieties of peas, lentils, and chickpeas and research on productivity and quality issues	Prof. Fred J. Muehlbauer, Grain Legume Genetics and Physiology Research Unit, USDA-ARS, 303 Johnson Hall, Washington State University, USA https://www.ars.usda.gov Email: muehlbau@wsu.edu

(continued)

Institution	Specialization and research activities	Contact information and website
International Center for Agricultural Research in the Dry Areas (ICARDA), Lebanon	CGIAR non-profit agricultural research institue, lentil genetic resources collection, conservation and introduction, genebank, climate change adaptation,	Dr. Shiv Kumar Agrawal, Lentil Breeder, Food Legumes Coordinator, ICARDA, Temporary HQs, Dalia Building 2nd Floor, Bashir El Kassar Street, Verdun, Beirut, Lebanon 1108-2010 https://www.icarda.org Email: sk.agrawal@cgiar.org
Indian Institute of Pulses Research, India	Research institutes, research on genetics, plant breeding and seed science technology, cyto-genetics and molecular breeding, plant genetic resource management and quality seed production.	Dr. Jitendra Kumar, Principal Scientist, Lentil Breeder, Crop Improvement Division, Indian Institute of Pulses Research, Kalyanpur-Kanpur-208024, India http://iipr.res.in/cimprovment.html Email: jitendra73@gmail.com
National Bureau of Plant Genetic Resources (NBPGR), India	Research institutes, national genebank, policy making, germplasm registration, conservation and distribution, crop improvement and cultivar development	Dr. Mohar Singh, Senior Scientist (Plant Breeding)ICAR-NBPGR Regional Station - Shimla Phagli, Shimla - 171004, Himachal Pradesh Pradesh, India http://www.nbpgr.ernet.in Email: mohar.singh2@icar.gov.in
Akdeniz, The University, Turkey	Teaching and research institute, genetics and plant breeding, mutation breeding of pulses, crop improvement	Prof. Cengiz Toker, Department of Field Crops, Faculty of Agriculture, Akdeniz, The University, Antalya, Turkey. http://ziraat.akdeniz.edu.tr/ Email: toker akdeniz.edu.tr
University of Birmingham, UK	Teaching and research institute, genetic conservation, in situ and ex situ conservation, global agrobiodiversity conservation, taxonomy and classification	Dr. Nigel Maxted, Senior Lecturer, School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK https://www.birmingham.ac.uk Email: n.maxted@bham.ac.uk
University of Córdoba (UCO) and Instituto de Agricultura Sostenible (CSIC), Spain	Teaching and research institute, plant genetic improvement, crop biodiversity, molecular genetic markers, crop protection, sustainable agriculture	Prof. José Ignacio Cubero, Professor Emeritus of the University of Córdoba, Researcher, Departamento de Mejora Genética Vegetal, Instituto de Agricultura Sostenible (CSIC),Apartado, Córdoba, Spain. http://www.ias.csic.es; www.uco.es Email: ge1cusaj@uco.es

Appendix II: Genetic Resources of Lentils

Cultivar	Important traits	Cultivation location
Noori,KLS 218,HUL 57,IPL 406,Masoor 93, DPL 62,L4076	Short duration, higher yields, rust resistance, high efficiency of zinc accumulation, bold size	India
Shiraz 96,NIAB Masoor 02,Masoor 04,NIAB Masoor 06,Manserha 89	Higher yields, rust resistance, high efficiency of zinc accumulation, short duration	Pakistan
Barimasur-1, Barimasur-2; Barimasur-3; Barimasur-4	High-yielding, resistance to rust	Bangladesh
Sikhar, Khajura Masuro 1, Khajura Masuro 2, Shital	Higher yields, rust resistance, high efficiency of zinc accumulation, short duration	Nepal
Sazak 91, Kayi 91, Pul 11, Yesil 21, Yeril Kirmizi, Kirmizi 51, Sakar	High yield, good weed resistance, seed size	Turkey
Bakria, Bichette, Hamira	Rust resistant, high yields	Morocco
Adaa, Alemaya, Alemtena, Teshale	Higher yields, rust resistance, wilt root rot resistance, high efficiency of zinc accumulation	Ethiopia
Nipper, Boomer	Resistance to botrytisgray mold and *Ascochyta* blight	Australia
Eston; Pardina, Morton	Small red types, medium-sized, yellow, small brown types, yellow cotyledons, very high yield	USA
Idlib 5	High yielding, fusarium wilt resistant	Syria
CDC Milestone, Glamis, Grandora, Sovereign, Vantage, Robin	*Ascochyta* blight resistant, high yield	Canada
Rajah	Red lentil, *Ascochyta* blight resistant	New Zealand

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Chapter 10
Mung Bean (*Vigna radiata* (L.) R. Wilczek) Breeding

Jungmin Ha and Suk-Ha Lee

Abstract Mung bean (*Vigna radiata* (L.) R. Wilczek) is a fast-growing, warm-season pulse crop that is primarily cultivated in developing countries in Asia. This crop has been showing a steady increase in production worldwide. Mung bean represents a good source of protein and contains higher folate and iron levels than other legumes. Moreover, it has a short lifecycle and can fix atmospheric nitrogen through symbiosis with nitrogen-fixing bacteria, making it ideal for intercropping with other major crops. Despite the importance of mung bean, there has been relatively little effort aimed at developing a breeding system for this crop, and genomic information is lacking compared to other legume species. Since mung bean has a small genome size, a short lifecycle, and is self-pollinating, it could be used as a model organism for studying legume plants. Moreover, the mung bean genome has recently been sequenced. The success of mung bean breeding depends on mining useful alleles from diverse germplasm and identifying markers closely associated with desirable phenotypes. The increasing affordability of high-throughput marker genotyping and the availability of a reference genome sequence will allow researchers and breeders to pinpoint the exact locations of genes and mutations that contribute to target phenotypes. Several research institutes and universities are currently constructing germplasm collections to maintain and secure mung bean genetic resources. Breeding via induced mutations and genetic engineering has helped improve mung bean cultivars, and genomic information from other well-studied legume species has been used to make up for the shortage of genomic information for mung bean. This chapter summarizes the current status of mung bean breeding, as well as genetic and genomic studies of this important crop.

Keywords Mung bean · Biodiversity · Domestication · Developing countries · Translational genomics · Quantitative trait locus · Synteny

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10.1 Introduction

10.1.1 Botanical Classification and Distribution

Mung bean is a fast-growing, warm-season pulse crop belonging to the subgenus *Ceratotropis* of the genus *Vigna* in the papilionoid subfamily of the Fabaceae. This self-pollinating diploid crop has a chromosome number of 2n = 2x = 22 (Arumuganathan and Earle 1991). Mung bean is mainly cultivated in South, East, and Southeast Asia by smallholder farmers. Mung bean grows in frost-free areas within a wide range of latitudes from Asia to Africa, South America, and Australia (Nair et al. 2012). With a cultivation area of approximately 6 million hectares, Asia has the largest mung bean cultivation area, with India, China, Myanmar, Thailand, Sri Lanka, Bangladesh and Indonesia, accounting for ~90% of world production (Lambrides and Godwin 2007). Among the Asian countries, India is the world's largest producer of mung bean, accounting for over 50% of global annual production (Nair et al. 2012).

10.1.2 Importance

Mung bean represents a good source of dietary protein and has higher folate and iron contents than most other legumes (Keatinge et al. 2011). Mung bean roots fix atmospheric nitrogen via symbiosis with nitrogen-fixing rhizobia, leading to improved soil fertility and texture, making this plant valuable both economically and nutritionally (Graham and Vance 2003). Intercropping mung bean in rice-rice and rice-wheat systems increases the yields of subsequently planted cereal crops and reduces pest occurrence, as it improves soil quality and reduces the amount of nitrogen fertilizer required in the soil due to its residual effects (Faria et al. 1989; Yaqub et al. 2010). Mung bean can be consumed in the form of vegetable sprouts or cooked as an ingredient in soups, porridge, pancakes, noodles, ice cream or sweet paste for cake fillings, making it highly versatile for the human diet. In addition, mung bean forage is beneficial in the diet of sheep, without any negative effects, and the haulm is used as livestock feed (Agboola and Fayemi 1972; Garg et al. 2004). Consequently, the global consumption of mung bean increased by 22–66% from 1984 to 2006, and annual production has increased by a large percentage (Shanmugasundaram et al. 2009).

Mung bean is currently regarded as a major cash crop and has therefore attracted interest by the research community worldwide. Thus, efforts are underway to develop an international mung bean network to coordinate research activities among different research groups.

10.1.3 Domestication, Selection, and Early Improvements

Mung beanis believed to have been domesticated in India ~3500 years ago based on domesticated mung bean diversity data, morphological studies, and archeological evidence (Fuller and Harvey 2006; Jain and Mehra 1980; Singh et al. 1975; Vishnu-Mittre 1974). However, the wild form of mung bean, *Vigna radiata* var. *sublobata*, is indigenous to the subtropical and tropical regions of northern and eastern Australia and is widely distributed throughout Africa, Asia, and Australia (Lawn and Cottrell 1988). Based on studies of protein variation and enzyme diversity, mung bean in West Asia exhibits the greatest variation, and mung bean is presumed to have moved to other Asian countries and to Africa (Tomooka et al. 1992a; Dela Vina and Tomooka 1994). Therefore, modern mung bean cultivars have resulted from multiple rounds of domestication, and this plant is currently distributed throughout southern and eastern Asia, Africa, and Austronesia (Lambrides and Godwin 2007).

10.2 Cultivation and Traditional Breeding

10.2.1 Current Cultivation Practices

Mung bean is a short-day, warm-season crop. This crop grows for 90–120 days from planting to maturity during the warm season without frost conditions (Oplinger et al. 1990). The flowering of mung bean responds differentially according to day length. Short days hasten flowering, whereas long days delay flowering (Aggarwal and Poehlman 1977). Mung bean seeds require temperatures of at least 15 °C for planting, and the optimum temperature for growing mung bean is a mean temperature of 20–30 °C during the crop production period. Elevations should not exceed 1800–2000 m (Oplinger et al. 1990). Mung bean is mainly grown in semiarid to subhumid lowland tropics and subtropics with 600–1000 mm of annual rainfall. If water stress occurs during the reproductive stage, it has a negative impact on flower formation, leading to a decrease in total yields (Raza et al. 2012). High humidity and excessive rainfall can result in disease problems and low yields (Oplinger et al. 1990). Mung bean grows well under good drainage conditions in sandy loam rather than clay soil, with a pH of 6.3–7.2 (Oplinger et al. 1990). Mung bean can be sown at various row spacing, from 20 to 100 cm, and narrower rows can have potential yield benefits. In plants grown in narrower rows, the nitrogen fixation rate is 15–30% higher than in those grown in broad rows, and faster ground cover of narrow spacing can help suppress weeds (Taj et al. 2002).

10.2.2 Current Agricultural Problems and Challenges

The harvesting index of mung bean is low due to its indeterminate growth habit, late and nonsynchronous maturity, and losses due to abiotic and biotic stresses (Alam Mondal et al. 2011; Fernandez and Shanmugasundaram 1988). A major problem in mung bean cultivation is synchronicity. Mung bean has an indeterminate growth habit, and flowering and pod maturity do not occur at a uniform time but are typically spread out over a long period (Khattak et al. 2001; Tah and Saxena 2009). If harvesting is performed once at the peak of the early harvest period, a large portion of the yield potential is lost because the harvest accounts for only ~50% of the total yield that could be harvested. However, delaying harvest can also lead to yield loss because mature and dried pods may shatter or fall off, and are more likely to be exposed to pest and pathogen attack at this stage. Preventing yield loss by performing multiple harvests also has its challenges, as it results in additional costs, and each harvest must be performed with care to avoid damaging the plants, which could make the harvesting procedure and the use of mechanical tools inefficient (Iqbal et al. 2015).

Synchronous maturity is a primary objective of mung bean breeding programs, as it could contribute greatly to productivity and cost-effective harvesting. Although early and even pod maturity were shown to have a positive effect on grain yield, the genetic basis of this trait in mung bean is currently unknown (Afzal et al. 2003; Chen et al. 2008).

10.2.3 Traditional Breeding

The objectives of conventional mung bean breeding include high yields, uniform maturity, and resistance to *Cercospora* leaf spot, powdery mildew, mung bean yellow mosaic virus, bruchids, bean flies and mung bean pod borer (Tomooka et al. 2005). In general, wild species serve as sources of useful genes because the currently cultivated germplasm has a limited number of alleles, as many alleles have been lost due to a genetic bottleneck that has occurred during domestication and modern breeding programs (Hyten et al. 2006). Useful alleles from wild relative species have been used to improve modern mung bean cultivars (Doyle 1988; Kumar et al. 2011; Tanksley and McCouch 1997). For example, a bruchid-resistant mung bean cultivar has been developed by importing an allele from a wild mung bean relative, TC1996, which is completely resistant to bruchid beetles, *Callosobruchus chinensis* and *C. maculatus*; these pests result in serious yield losses during mung bean storage (Somta et al. 2008; Talekar 1988; Tomooka et al. 1992b). A yellow mosaic disease-resistant allele from *Vigna mungo*, a wild relative species, was transferred into cultivated mung bean and used to develop yellow mosaic virus-resistant mung bean cultivars (Basak et al. 2005; Gill et al. 1983;

Singh 1980). Genetic maps have been constructed based on cultivated mung bean and wild mung bean accessions or related wild species, providing genetic information about agronomically-important traits such as seed quality, weathering tolerance and pest/disease resistance (Lörz and Wenzel 2007; Isemura et al. 2012; Lambrides et al. 2000; Wang et al. 2016). Since the importance of maintaining germplasms has become increasingly clear, several research institutions and universities are currently constructing germplasm collections to sustain mung bean genetic resources. AVRDC-The World Vegetable Center, Tainan, Taiwan, currently holds the world's largest *Vigna* germplasm collection, consisting of 11,832 accessions (10,673 *Vigna* species, 881 *V. angularis*, 278 *V. unguiculata*), representing important resources for interspecific hybridization for mung bean cultivar improvement.

10.3 Germplasm Biodiversity and Conservation

10.3.1 Germplasm Diversity

The general goal of breeding is to accumulate useful alleles from various parental lines into a new plant variety. The first step in finding superior alleles or the individuals carrying them is to secure a germplasm pool with high genetic diversity. Essentially, breeders have to rely on existing natural DNA variation because DNA modification using genetic engineering is still limited and is even viewed unfavorably by the general public (Priest 2000). Therefore, the availability of natural genetic resources with rich variation is fundamental for successful breeding programs. Therefore, many institutes have been established for various research activities including mung bean germplasm conservation and cultivar improvement.

Mung bean germplasm is maintained at several centers throughout the world, including AVRDC-The World Vegetable Center, Taiwan; National Bureau of Plant Genetic Resources of the Indian Council of Agricultural Research; the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences; the Plant Genetic Resources Conservation Unit of the University of Georgia, USA and the University of the Philippines. Additionally, the Rural Development Administration (RDA), Korea, and the University of the Philippines house duplicates of some of the mung bean germplasm found in AVRDC-The World Vegetable Center (Ebert 2013; Kim et al. 2015). Mung bean core collections have also been established in China, India, Korea and the USA to allow breeders and researchers to have easier access to useful germplasm and to enable the efficient use of genetic resources. A core collection consisting of 1481 accessions and a mini-core collection consisting of 296 accessions were constructed by AVRDC-The World Vegetable Center based on phenotypic and molecular characterization using 20 SSR markers, respectively (Shanmugasundaram et al. 2009). Due to the importance of genetic

diversity, molecular markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers have long been used to analyze germplasm and genetic diversity. These techniques have been applied to improve mung bean cultivars with a focus on yield, nutritional improvement, and disease resistance through linkage map construction.

10.3.2 Cultivar Characterization and Phylogeny

Kang et al. (2014) recently sequenced cultivated mung bean *Vigna radiata* var. VC1973A and obtained the transcriptome sequences of 22 *Vigna* accessions from 18 species (Table 10.1). Based on de novo transcriptome assembly, approximate divergence dates were calculated through phylogenetic analysis (Fig. 10.1). This phylogenetic study allowed the relationships of the two homoeologous genomes of the allotetraploid wild species, *V. reflex-pilosa*, to be traced. One genome was found to be closely related to *V. trinervia* (divergence date, 0.09 million years ago), and the other was found to be a sister to seven wild relatives (divergence date, 2.7 million years ago), suggesting that the diploid progenitor lineage has not been sampled or may be extinct. These studies have provided insights into the evolution within *Vigna* species, which may facilitate the improvement of mung bean cultivars.

10.4 Molecular Breeding

10.4.1 Molecular Marker-Assisted Breeding

The use of molecular marker systems has helped breeders identify loci associated with desirable phenotypes. Tracking the inheritance of a DNA segment with known benefits using molecular markers is more precise and efficient than traditional breeding (Collard and Mackill 2008). The recent availability of physical map data and the development of high-throughput marker genotyping based on high-coverage, whole-genome sequencing have facilitated forward genetics studies by increasing the resolution of physical maps and marker density within linkage groups (Huang et al. 2010; Zhou et al. 2015).

Restriction fragment length polymorphism (RFLP) markers were initially used to analyze the genetics of bruchid resistance in mung bean via forward genetics (Young et al. 1992). A total of 153 RFLP markers were grouped into 14 linkage groups covering 1295 centiMorgans (cM), with an average marker interval of 9.3 cM. Quantitative trait loci (QTLs) for seed weight were also identified (Fatokun et al. 1992). The initial linkage map of mung bean, consisting of 11 linkage groups,

Table 10.1 List of *Vigna* species subjected to transcriptome analysis

Scientific name	Common name	Accession or cultivar name	Origin	Number of chromosomes	Estimated genome size (Mbp)
Vigna mungo var. *mungo*	Blackgram	Chai Nat 80	Thailand	2n = 2x = 22	538
Vigna mungo var. *silvestris*	Wild blackgram	TC2210	India	2n = 2x = 22	538
Vigna radiata var. *radiata*	Mung bean	Sunhwanokdu	Korea	2n = 2x = 22	612
Vigna radiata var. *sublobata*	Wild mung bean	TC1966	Madagascar	2n = 2x = 22	465
Vigna angularis var. *angularis*	Adzuki bean	Kyungwonpat	Korea	2n = 2x = 22	539
Vigna umbellata	Rice bean	CIAT34386	Unknown	2n = 2x = 22	562
Vigna umbellata	Wild rice bean	2004 T2	Thailand	2n = 2x = 22	562
Vigna reflexo-pilosa var. *glabra*	Créole bean	V1160	The Philippines	2n = 4x = 44	978
Vigna reflexo-pilosa var. *reflexo-pilosa*	Wild creole bean	AusTRCF100879	Papua New Guinea	2n = 4x = 44	978
Vigna aconitifolia	Moth bean	AusTRCF96939	India	2n = 2x = 22	1100
Vigna trilobata	NA	TVNu-953	India	2n = 2x = 22	513
Vigna stipulacea	NA	ILRI524	India	2n = 2x = 22	NA
Vigna hainiana	NA	AusTRCF85155	India	2n = 2x = 22	1394
Vigna grandiflora	NA	JP108509	Thailand	2n = 2x = 22	NA
Vigna subramaniana	NA	NI1135	India	2n = 2x = 22	NA
Vigna nakashimae	NA	KUV12	Unknown	2n = 2x = 22	NA
Vigna nepalensis	NA	AusTRCF85148	India	2n = 2x = 22	NA
Vigna riukiuensis	NA	96 J028	Japan	2n = 2x = 22	NA
Vigna minima	NA	JP120064	Thailand	2n = 2x = 22	1027
Vigna trinervia var. *trinervia*	NA	AusTRCF319618	Papua New Guinea	2n = 2x = 22	NA
Vigna subterranea var. *subterranea*	Bambara groundnut	TVSu-84	Nigeria	2n = 2x = 22	880
Vigna vexillata var. *macrosperma*	Zombie pea	NI339	Costa Rica	2n = 2x = 22	562

Source: Kang et al. (2014)

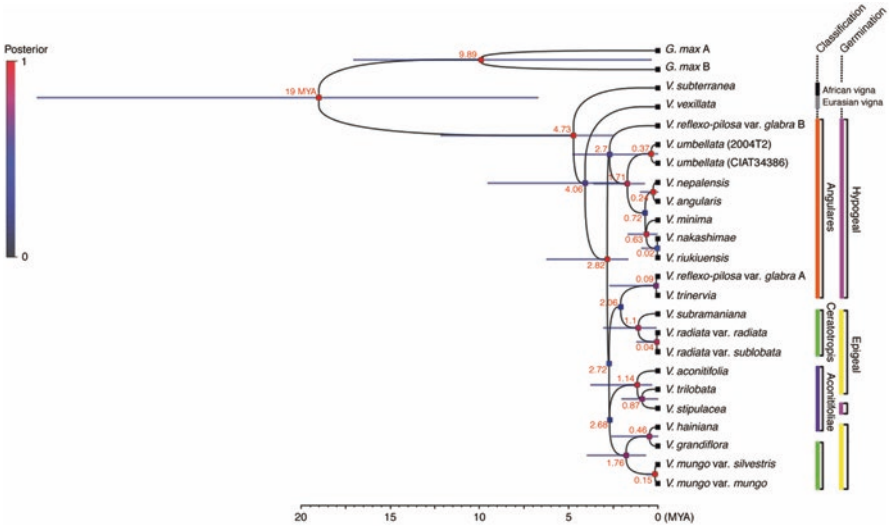

Fig. 10.1 Phylogenetic tree constructed based on de novo transcriptome assemblies from 22 *Vigna* species. (Source: Kang et al. 2014)

was developed using RFLP markers from mung bean and an interspecific hybrid population generated between *Vigna radiata* ssp. *radiata* and *V. radiata* ssp. *sublobata.* The map consisted of 171 markers covering 1570 cM, with an average marker interval of 9 cM. Several important traits were mapped, such as seed size and resistance to powdery mildew and seed bruchids (Menancio-Hautea et al. 1992).

The first random amplified polymorphic DNA (RAPD) study in mung bean was conducted to evaluate the genetic diversity among 23 accessions of wild and cultivated mung bean species including *Vigna angularis*, *V. umbellata*, *V. radiata*, *V. aconitifolia* and *V. mungo* (Kaga et al. 1996). By integrating 52 RFLP and 56 RAPD markers, a genetic map comprising 12 linkage groups was constructed from an F2 mapping population from a cross between *V. radiata* ssp. *radiata* and *V. radiata* ssp. *sublobata* (Lambrides et al. 2000). In addition, a genetic map consisting of 115 markers, covering 691.7 cM, was constructed using a recombinant inbred line derived from the previously examined F2 population. In addition, genes responsible for bruchid resistance were mapped to a linkage map constructed using both RFLP and RAPD markers (Kaga and Ishimoto 1998). A genetic map with higher marker density was subsequently constructed using RFLP markers alone (Humphry et al. 2002). Genetic diversity in mung bean was then assessed using RAPD and inter simple sequence repeat (ISSR) markers (Chattopadhyay et al. 2005).

Simple sequence repeat (SSR) markers are highly informative markers that are codominant, PCR-based, easy to generate and highly polymorphic in terms of repeat-length. The first SSR markers reported in mung bean were generated from 6 SSR sequences with 5 different types of motifs (Yu et al. 1999). Based on the

close phylogenetic relationship between mung bean and adzuki bean, SSR markers from adzuki bean were used to evaluate genetic diversity in 415 cultivated, 189 wild and 11 intermediate mung bean accessions, and higher allelic polymorphisms were successfully detected in wild mung bean (Sangiri et al. 2008). Using partial linkage maps, SSR markers associated with resistance to powdery mildew and *Cercospora* leaf spot were identified (Chankaew et al. 2011; Kasettranan et al. 2010). Using 237 SSR markers from mung bean, cowpea, adzuki bean and common bean, and 193 expressed sequence taq (EST)-SSR markers from soybean, the 11 initial linkage groups were constructed, covering 727.6 cM (Isemura et al. 2012). In total, 105 QTLs and genes related to 38 domestication-related traits were identified. The positions of previously mapped genes and QTLs, such as the bruchid resistance gene, *Br1*, 100-seed weight QTLs and the gene controlling black mottle on the seed coat were corrected on the genetic map (Fatokun et al. 1992; Kaga and Ishimoto 1998; Lambrides et al. 2000).

Before the advent of next generation sequencing (NGS) and high-throughput genotyping, the number of available polymorphic genetic markers represented a bottleneck to quantifying the genetic diversity of a population. The use of a limited number of markers can introduce bias in QTL studies because the sampled sequences may not represent the allelic diversity of the whole genome (Moragues et al. 2010). Due to advancements in NGS, researchers have focused on finding single nucleotide polymorphisms (SNPs) to be used as genetic markers. SNP markers are single base, biallelic, codominant, and ubiquitous over the genome (Brumfield et al. 2003). Two mung bean cultivars, Seonhwanogdu and Jangannogdu, were sequenced using the Illumina 454 sequencing platform to study resistance to stink bug (*Riptortus clavatus*) and adzuki bean weevil (*Callosobruchus chinensis*) (Moe et al. 2011). By comparing de novo assembled contigs from the two cultivars, 1334 and 1630 microsatellite repeat motifs, respectively, were identified, and 2098 single nucleotide variations were detected. A number of markers developed in this study have served as valuable resources for functional genomics studies by increasing the marker density of linkage maps. Cultivars Seonhwanogdu and Gyeonggijaerae5 were sequenced on the Illumina HiSeq2000 platform, and 265,001 homozygous SNPs were found.

These sequence variations identified in several mung bean cultivars can be analyzed in the context of their physical locations in the genome if a reference genome sequence is available. Seonhwanogdu (*VC1973A*), its polyploidy relative *Vigna reflexo-pilosa* var. *glabra* (accession *V1160*), and its wild relative *V. radiata* var. *sublobata* (accession *TC1966*) were sequenced and de novo assembled in 2014, and the reference genome sequence of mung bean was published (Kang et al. 2014). Along with the draft genome assembly, transcriptome assemblies from 22 accessions of 18 *Vigna* species were analyzed, facilitating genomic research in the subgenus *Ceratotropis* and providing insight into the evolution of *Vigna* species. In total, 2748 scaffolds covering 431 Mbp were anchored onto 11 pseudochromosomes using a high-density genetic map. This genetic map was constructed using 1321

SNP markers developed by genotyping-by-sequencing (GBS) from an F6 population of 190 recombinant inbred lines (RILs) derived from a cross between Seonhwanogdu (*VC1973A*) and Gyeonggijaerae5 (V2985). The N50 length is 1.62 Mbp, and ~80% of the estimated genome size (579 Mbp) is covered in this map. In total, 22,427 high-confidence protein-coding genes were annotated. Compared to the previous low-resolution linkage maps and fragmental genomic sequence information, this study represents an important milestone in *Vigna* genomic analysis. In total, 2,922,833 SNPs were revealed between wild and cultivated mung bean varieties across the genome at a frequency of 6.78 per 1 kbp. Among these SNPs, 63,294 are located in protein-coding sequence (CDS) regions, 30,405 of which represent nonsynonymous changes. Also, 55,689 of 342,853 insertions/deletions (InDels) are located around genic regions, resulting in frameshifts in 1057 genes. Microsatellite repeats (200,808 SSRs) were detected, which could possibly be used as SSR markers (Kang et al. 2014).

10.4.2 Functional Genomics

Molecular markers developed from transcriptome data are also highly informative because they are based on variations present in expressed regions of the genome. Since expressed sequence tag (EST) sequences for many crop species have been deposited in databases, data mining is a fast, cost-effective way to develop markers. In recent years, EST-based SSR markers have been developed for functional genomics studies in mung bean. Using 12,596 EST sequences from cv. Jangannogdu, 2299 SSR motifs were identified in 1848 EST sequences from which 97 PCR primer sets were designed and successfully amplified in two mung bean cultivars, TM96-2 and TARM-18 (Moe et al. 2011). Approximately 45% and 55% of the SSR motifs are located in CDS and untranslated regions (UTRs), respectively. Through data mining of the NCBI database for mung bean, EST-SSR markers were identified without incurring additional costs for sequencing (Chavan and Gacche 2014). Wang et al. (2015) used biotin-labeled oligo-probes and streptavidin-coated beads to construct an SSR-enriched library from six mung bean genotypes (ACC41, VC1973A, V2709, C01478, C01558, C01579) and discovered 308,509 SSR motifs. To characterize and validate SSR markers detected from in silico EST-SSR markers, the mung bean transcriptome was sequenced using Illumina paired-end sequencing (Chen et al. 2015). Putative SSR markers were identified by analyzing repetitive sequences in the assembled unigenes, and 13,134 EST-SSRs were detected. Among the primers designed from randomly chosen EST-SSRs, 66 primers were successfully amplified and found to be polymorphic among 31 mung bean accessions. By annotating the unigenes harboring SSRs verified by PCR, the possible effects of EST-SSRs were identified. These recent studies have resulted in the development of a number of SSR markers, thus resulting in advances in linkage map and QTL mapping analysis, which could further facilitate mung bean breeding programs.

10.4.3 Translational Genomics

Genomic information from well-studied species can be used to analyze other species; this concept is referred to as translational genomics (Varshney et al. 2015). By applying genomic information from model species to crops that are poorly understood, translational genomics has helped breeders and researchers improve and study various crops more easily (Stacey and VandenBosch 2005). Genomic sequences are currently available for mung bean, but few QTL studies have been performed. Several studies have been performed to characterize the mung bean genome using translational genomics. Flowering genes in mung bean have been identified using genome-wide comparisons between mung bean and *Arabidopsis* (Kim et al. 2014). In *Arabidopsis*, 207 genes are known to be involved in flowering, 129 of which are homologous to mung bean genes. Some of these genes are located close to SSR markers on a genetic map previously constructed for mung bean (Isemura et al. 2012). In addition, by comparing the mung bean and soybean genomes, five putative flowering-related genes in mung bean were found to be homologous to soybean flowering genes (Kim et al. 2014).

Comparative analysis between mung bean and soybean would facilitate analysis of mung bean, as SoyBase (the USDA-ARS soybean genetic database) lists over 1000 QTLs associated with more than 100 agronomically-important traits, such as seed weight, days to first flowering, seed oil content, plant height and so on. Based on sequence similarity and conserved synteny between soybean and mung bean, 1089 putative mung bean QTLs were identified using marker sequence information associated with QTLs in soybean (Table 10.2; Fig. 10.2) (Kim et al. 2015). For example, synteny blocks in soybean containing QTLs for seed weight and nematode resistance are homologous to synteny blocks in mung bean harboring QTLs for seed size/germination and bruchid resistance (Fig. 10.3) (Kang et al. 2014).

Table 10.2 Putative mung bean QTLs identified through analyzing sequence similarity and conserved synteny between soybean and mung bean

Trait	Soybean QTLs	Mung bean QTLs
First flower	104	54
Leaflet length	66	53
Leaflet width	61	55
Plant height	268	171
Pod maturity	196	142
Pod number	59	40
Seed oil	236	178
Seed oil to protein ratios	16	0
Seed protein	356	140
Seed weight	272	245
Seed weight per plant	16	11

Source: Kim et al. (2015)

Fig. 10.2 Identification of putative QTLs in mung bean using translational genomics. (Source: Kim et al. 2015)

Another synteny analysis identified a candidate gene for QTLs found in mung bean (Fig. 10.4). A mung bean genomic region containing a QTL for both days-to-flowering and days-to-first-flowering has a syntenic relationship with a soybean genomic region containing QTLs for first flowering that mapped to key flowering genes encoding phytochrome A. Through this comparative analysis, a candidate flowering gene was identified in mung bean, as well as QTLs for flowering (Hwang et al. 2017). To date, a few QTL studies involving the identification of putative candidate genes for these QTLs have been conducted in mung bean. With the availability of the mung bean reference genome sequence, translational genomics studies could be performed, which would facilitate molecular studies in mung bean leading to functional characterization of the genes of interest.

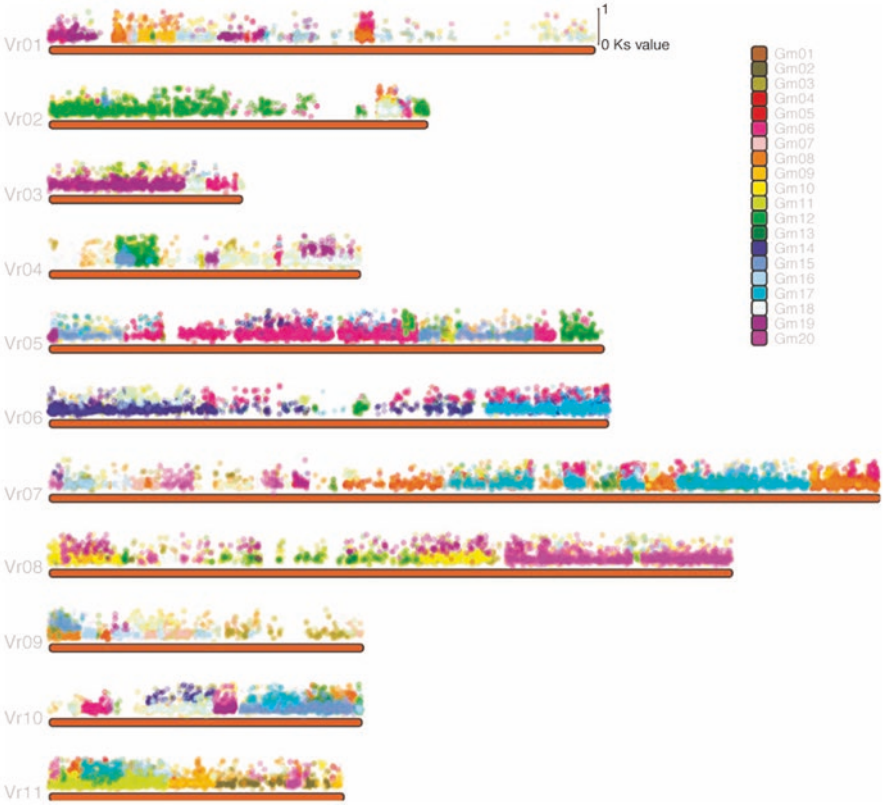

Fig. 10.3 Distribution of synteny blocks between mung bean and soybean on the mung bean chromosomes. (Source: Kang et al. 2014)

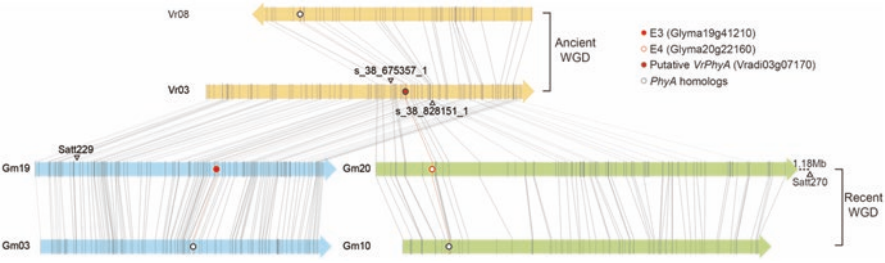

Fig. 10.4 Identification of a candidate gene in a QTL region using translational genomics. (Source: Hwang et al. 2017)

10.5 Genetic Engineering

10.5.1 Limitations of Conventional Breeding

Efforts to develop new mung bean varieties via traditional breeding have achieved limited success due to the narrow genetic variation in this crop, as mung bean is a self-pollinated species, and only a few parental lines have been used repeatedly in breeding programs. In addition to increasing genetic diversity using wild relatives as breeding materials, the use of biotechnological tools for improving mung bean cultivars has emerged as a powerful way to overcome bottlenecks in mung bean breeding, as these tools enable key genes to be introduced into elite plant lines.

10.5.2 Regeneration in Mung Bean

The ability to regenerate whole plants through tissue culture allows genes to be transferred into plant cells, followed by regeneration to produce stably transformed plants (Chandra and Pental 2003). Progress in transgenic research in mung bean has been very slow due to its highly recalcitrant nature in tissue culture and its very low frequency of regeneration, especially after transformation (Dita et al. 2006; Eapen 2008; Varshney et al. 2015). Although several research studies have reported regeneration protocols for mung bean via embryogenesis (Kaviraj et al. 2006; Sivakumar et al. 2010) and organogenesis (Gulati and Jaiwal 1994; Himabindu et al. 2014; Rao et al. 2005), the regeneration efficiency is very low, except for a few reports using cotyledonary node explants (Amutha et al. 2006; Sagare and Mohanty 2015; Vijayan et al. 2006; Yadav et al. 2010). To date, there are only a few reports of the production of stably transformed mung bean where whole transgenic plants have been recovered and transgenes have been stably inherited and passed to subsequent generations (Baloda et al. 2017; Mahalakshmi et al. 2006; Sonia et al. 2007; Vijayan and Kirti 2012; Yadav et al. 2012).

10.5.3 Genetic Transformation

Genetic transformation in mung bean was first conducted using hypocotyls and primary leaves (Jaiwal et al. 2001). A binary vector containing the reporter gene *GUS* and the selection marker *nptII* was successfully transformed into mung bean. Mahalakshmi et al. (2006) developed a genotype-independent, high-frequency plant regeneration protocol for mung bean with a survival rate of ~90% and successfully transformed primary leaf explants. Subsequently, the insecticidal α-amylase inhibitor-1 gene from *Phaseolus vulgaris* and the bialaphos resistance (*bar*) gene were successfully expressed in mung bean using cotyledonary node explants (Sonia et al. 2007). After introducing a pathogenesis-related gene (*BjNPR1*) from mustard into

mung bean, transgenic mung bean plants showed resistance against fungal-related diseases (Vijayan and Kirti 2012). Yadav et al. (2012) transformed the *annexin 1 bj* gene into mung bean and the resulting transgenic plants showed improved drought stress tolerance. Transgenic mung bean plants with increased salt tolerance were obtained by introducing the *codA* gene into mung bean (Baloda et al. 2017).

Despite its recalcitrant nature in tissue culture and its low regeneration frequency, researchers have developed protocols for regenerating and transforming mung bean at high frequency. Although DNA modification of food crops is viewed negatively by consumers, genetic engineering can help save time and labor, thereby overcoming the limitations of traditional breeding. Advances in the development of biotechnological tools, such as clustered regularly interspaced short palindromic repeats (CRISPR) Cas9/dCas9, and their successful application to food crops might help mitigate public concerns while improving mung bean cultivars (Ran et al. 2013).

10.6 Mutation Breeding

10.6.1 Mutagenesis and Genetic Diversity

Conventional breeding methods have limited effects in enhancing yields in mung bean due to their low genetic variability within the existing germplasm. There has been a continuous decline in the genetic diversity of this crop, prompting breeders to induce mutations artificially. Inducing mutations using physical and chemical mutagens can be one of the most effective ways to create genetic variability. This technique has played a key role in modern plant breeding and genetic studies (Raina et al. 2016).

10.6.2 Cultivars Developed by Mutagenesis

Several mutagens have been used in mung bean, including ethyl methane sulfonate (EMS), sodium azide (SA), hydrazine hydrate (HZ) and gamma rays. Significant increases in the number of fertile branches and pods as well as seed yields were detected in mutant mung bean obtained through the use of EMS and HZ (Wani 2006). Several mung bean cultivars were treated with EMS and gamma rays, thereby generating genetic variability and leading to the development of new cultivars with high yields and increased resistance to bean fly infestation (Khan and Goyal 2009; Wani 2006). Mutants with variegated leaves or synchronous pod maturity were obtained through the use of gamma irradiation (Sangsiri et al. 2007; Tah and Saxena 2009). SA, EMS, and gamma rays were used to produce a wide range of viable morphological and physiological mutants (Auti and Apparao 2009). To date, 39 mutant mung bean cultivars have been officially released, including cultivars derived from crossing with mutant varieties (Table 10.3). The availability of these varieties

Table 10.3 List of mutant *Vigna radiata* (L.) Wil. varieties

Variety	Country	Local registration year	Mutagen	Characteristics
Dhauli (TT9E)	India	1979		High yield, early maturity, tolerance or resistance to MYMV
Pant Moong 2	India	1982	Irradiation with gamma rays	Resistance to MYMV, more pods, high yield
Co 4	India	1982	Irradiation of seeds with gamma rays	High yield, early maturity, resistance to drought
ML 26-10-3	India	1983	Irradiation of seeds with gamma rays	Resistance to MYMV, high yield
TAP-7	India	1983	Irradiation of seeds with gamma rays	Early maturity, resistance to mildew and leaf spot, high yield
NIAB Mung-28	Pakistan	1983	Irradiation of seeds with gamma rays	Early and uniform maturity, high yield
NIAB Mung 19-19	Pakistan	1985	Irradiation with gamma rays	Early maturity, determinate type, high yield, tolerance to MYMV
NIAB Mung 121-25	Pakistan	1985	Irradiation of seeds with gamma rays	Early maturity, determinate type, high yield
NIAB Mung 13-1	Pakistan	1986	Irradiation of seeds with gamma rays	Early maturity, shortness, more pods, harvest index, seed size, high yield
NIAB Mung 20-21	Pakistan	1986	Irradiation of seeds with gamma rays	Early maturity, shortness, harvest index, high yield, tolerance to MYMV, resistance to *Cerospora* leaf spot
Camar	Indonesia	1987	Irradiation of seeds with gamma rays	Resistance to *Cerospora* leaf spot, resistance to *Uromyces* sp., resistance to scrab diseases, high yield, tolerance to salinity and acid soil
NIAB Mung 54	Pakistan	1990	Irradiation with gamma rays	Early and synchronous maturity, non-shattering pods, tolerance to MYMV and CLS, large seed size, high yield
NIAB Mung 51	Pakistan	1990	Irradiation with gamma rays	Early and synchronous maturity, non-shattering pods, profuse hairiness, tolerance to MYMV and CLS, large seed size, high yield
Binamoog-1	Bangladesh	1992		

(continued)

Table 10.3 (continued)

Variety	Country	Local registration year	Mutagen	Characteristics
MUM-2	India	1992	Treatment with EMS	High yield, resistance to diseases
BM 4	India	1992		High yield, early maturity, tolerance or resistance to MYMV
NIAB Mung 92	Pakistan	1992	Hybridization with mutant NIAM Mung 36	Resistance to MYMV, early maturity, resistance to grain shattering, large seed size
LGG 450	India	1993		High yield, early maturity, tolerance or resistance to MYMV
LGG-407	India	1993		High yield, early maturity, tolerance or resistance to MYMV
Binamoog-2	Bangladesh	1994	Hybridization with gamma-ray induced mutant MB-55(4)	Large seed size, early and synchronous maturity, high yield, tolerance to leaf MYMV and *Cercospora* leaf spot
TARM-2	India	1994	Hybridization with a mutant RUM 5 obtained by gamma irradiation	High yield, medium late maturity, resistance to powdery mildew disease
TARM-18	India	1996	Hybridization with a mutant variety TARM-2	High yield, resistance to powdery mildew disease
Binamoog-3	Bangladesh	1997	Irradiation of hybrid seeds from cross (Mutant MB55-4 x AURDC line V1560D)	Seed yield, synchronous pod maturity, tolerance to MYMV and *Cercospora* leaf spot
Binamoog-4	Bangladesh	1997	Irradiation of hybrid seeds from cross (Mutant MB55-4 x AURDC line V1560D)	Seed yield, synchronous pod maturity, early maturing, dwarf plant type, tolerance to MYMV and *Cercospora* leaf spot
TARM-1	India	1997	Hybridization with a mutant RUM 5 obtained by gamma irradiation	High yield, resistance to powdery mildew disease, medium maturity
Binamoog-5	Bangladesh	1998	Irradiation of hybrid seeds from cross (Mutant MB55-4 x AURDC line V1560D)	High seed yield, synchronous pod maturity, tolerance to MYMV and *Cercospora* leaf spot

(continued)

Table 10.3 (continued)

Variety	Country	Local registration year	Mutagen	Characteristics
NIAB Mung 98	Pakistan	1998	Hybridization with mutant variety Mung 20-21 obtained by gamma rays	Resistance to MYMV and *Cercospora* leaf spot, high yield, medium seed size
AEM-96	Pakistan	1998		
Chai Nat 72	Thailand	1999	Irradiation with gamma rays	High yield, large grain size, resistance to fungal disease
Binamoog-6	Bangladesh	2005	Gamma irradiation of an advanced mutant line, VC-6173-10	Increased pod, reduced seed size, tolerance to leaf MYMV and *Cercospora* leaf spot
Binamoog-7	Bangladesh	2005	Treatment of seeds from Binamoog-2 with EMS	High seed yield, synchronous pod maturity, tolerance to MYMV and *Cercospora* leaf spot
TMB-37	India	2005		Early maturity, resistance to MYMV, high yield, large seed size,
NIAB MUNG 2006	Pakistan	2006	Hybridization with mutant NIAB Mung 92	Purple hypocotyl and stem, high number of pods and clusters, resistance to diseases
TM-96-2	India	2007	Hybridization with mutant variety TARM-2 obtained by irradiation with gamma rays	Resistance to powdery mildew disease and *Corynespora* leaf spot, early maturity
TJM-3	India	2007	Hybridization with a mutant variety TARM-1 obtained by irradiation with gamma rays	Resistance to MYMV, powdery mildew and *Rhizoctonia* root-rot disease, early maturity, large seeds
TM 2000-2	India	2010		Resistance to powdery mildew
Binamoog-8	Bangladesh		Irradiation of seeds of advanced line MB-149 with gamma rays	Synchronous pod maturity
Binamoog-9	Bangladesh		Irradiation of seeds of BARI Mung-6 with gamma rays	Synchronous pod maturity
Chai Nut 84-1	Thailand			

Source: FAO/IAEA Mutant variety database, 2018 (https://mvd.iaea.org)

increases genetic diversity, and they provide breeding materials for conventional plant breeding, thus contributing to the genetic improvement of mung bean. The mutagens that were used to produce these varieties are not targeted to specific genetic regions, and the casual variations resulting from mutagenesis have not been identified. The availability of the mung bean reference genome combined with recent advances in targeted mutagenesis and sequencing techniques will enable researchers and breeders to identify the casual variations induced by mutagens, thereby facilitating forward genetics analysis to characterize the links between genes and phenotypes (Kang et al. 2014; Ran et al. 2013). Mutation-assisted plant breeding will play a crucial role in the generation of optimized crop cultivars to migrate the threats posed by global climate change and food shortages.

10.7 Diseases in Mung Bean

10.7.1 Impact of Pathogens

Numerous types of pathogens affect mung bean, including viruses, fungi, bacteria and nematodes. Diseases in mung bean can affect various tissues, including but not limited to seeds, leaves, flowers, roots and stems. Pathogens can reduce mung bean yields by affecting nearly all stages of development, including seed germination, shoot development, flower development and so on. These reduced yields can have a detrimental impact on the wellbeing of the many people dependent on mung bean. A complete list of pests that use mung bean as a major and minor host can be found at the Centre for Agriculture and Bioscience International (CABI, https://www.cabi.org/) (Tables 10.4 and 10.5). However, few cultivars have been bred for full resistance against such pathogens, and few studies have focused on the identification and characterization of pathogens of mung bean.

10.7.2 Viral Pathogens

One of the best-studied viruses in mung bean is the geminivirus mung bean yellow mosaic virus (MYMV). This virus is composed of two DNA components, DNA 1 and DNA 2, each comprising roughly 2.7 kb; the full reference genome for MYMV is currently available (Morinaga et al. 1993). The leaves of MYMV-infected plants show yellow discoloration. MYMV is the most devastating virus in mung bean, with yield losses of up to 85% (Karthikeyan et al. 2014). There are currently no fully-resistant cultivars available, and even highly resistant lines show high levels of variation, depending on the geographic location (Nair et al. 2017). MYMV is transmitted by the tobacco whitefly (*Bemisia tabaci* Genn.), a vector of many other geminiviruses as well, including other mung bean viral diseases such as mung bean

Table 10.4 List of pests that use mung bean as a major host (CABI, https://www.cabi.org/)

Pest	Details, common name	Pest	Details, common name
Acherontia styx	Small death's head hawkmoth	*Macrophomina phaseolina*	Charcoal rot of bean/tobacco
Agrius convolvuli	*Convolvulus* hawkmoth	*Maruca amboinalis*	
Alfalfa mosaic virus	Alfalfa yellow spot	*Medythia suturalis*	Striped bean flea beetle
Amrasca		*Megalurothrips distalis*	
Amrasca biguttula	Indian cotton jassid	*Megalurothrips usitatus*	Bean flower thrips
Amsacta moorei	Tiger moth	*Mung bean yellow mosaic virus*	
Aphis craccivora	Groundnut aphid	*Nezara viridula*	Green stink bug
Blackeye cowpea mosaic virus	BICMV	*Ophiomyia phaseoli*	Bean fly
Boeremia exigua var. *exigua*	Leaf spot	*Orgyia postica*	Cocoa tussock moth
Borreria latifolia	Broadleaf buttonweed	*Oxycetonia versicolor*	
Broad bean wilt virus	*Lamium* mild mosaic	*Phyllophaga*	White grubs
Cadophora gregata	Brown stem rot of soybean	*Pratylenchus brachyurus*	Root-lesion nematode
Callosobruchus	Pulse beetle	*Rhizobium radiobacter*	Crown gall
Callosobruchus analis	Weevil (bean)	*Rhizobium rhizogenes*	Gall
Callosobruchus chinensis	Chinese bruchid	*Riptortus*	
Callosobruchus maculatus	Cowpea weevil	*Riptortus clavatus*	Bean bug
Callosobruchus phaseoli		*Riptortus dentipes*	Pod-sucking bug
Chalara elegans	Black root rot	*Riptortus linearis*	
Choanephora cucurbitarum	*Choanephora* fruit rot	*Rottboellia cochinchinensis*	Itch grass
Colletotrichum dematium	Leaf spot	*Scirtothrips dorsalis*	Chilli thrips
Colletotrichum truncatum	Soybean anthracnose	*Sclerotinia sclerotiorum*	Cottony soft rot
Commelina benghalensis	Wandering Jew	*Spodoptera littoralis*	Cotton leafworm
Cowpea severe mosaic virus		*Spodoptera litura*	Taro caterpillar
Cucumber mosaic virus	Cucumber mosaic	*Stegobium paniceum*	Drugstore beetle
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	Bacterial tan spot	*Thanatephorus cucumeris*	Many names depending on host
Erysiphe diffusa	Soybean powdery mildew	Tomato spotted wilt virus	Tomato spotted wilt
Gonocephalum	False wireworm	Urd bean leaf crinkle virus	
Heterodera cajani	Pigeon pea cyst nematode	*Xanthium spinosum*	Bathurst burr
Lampides boeticus	Pea blue butterfly	*Xanthomonas campestris* pv. *vignaeradiatae*	

Table 10.5 List of pests that use mung bean as minor host (CABI, https://www.cabi.org/)

Pest	Common name
Acyrthosiphon pisum	Pea aphid
Aphis gossypii	Cotton aphid
Aproaerema modicella	Groundnut leaf miner
Aspergillus niger	Black mold of onion
Athelia rolfsii	Sclerotium rot
Bean common mosaic necrosis virus	
Bean common mosaic virus	Common mosaic of beans
Cleome rutidosperma	Fringed spiderflower
Cochliobolus lunatus	Head mold of grasses, rice and sorghum
Cochliobolus sativus	Root and foot rot
Colletotrichum capsici	Leaf spot of peppers
Colletotrichum lindemuthianum	Anthracnose of bean
Corcyra cephalonica	Rice meal moth
Cowpea aphid-borne mosaic virus	
Cuscuta campestris	Field dodder
Cyrtozemia dispar	
Dactyloctenium aegyptium	Crowfoot grass
Etiella zinckenella	Pea pod borer
Glomerella cingulata	Anthracnose
Haematonectria haematococca	Dry rot of potato
Heterodera glycines	Soybean cyst nematode
Hilda patruelis	Groundnut hopper
Holotrichia serrata	White grub
Hoplolaimus indicus	Lance nematode
Leveillula taurica	Powdery mildew of cotton
Melanagromyza obtusa	Pod fly
Melanagromyza sojae	Soybean stem miner
Meloidogyne ethiopica	Root-knot nematode
Meloidogyne incognita	Root-knot nematode
Olpidium brassicae	Lettuce big vein
Ophiomyia centrosematis	Stem fly
Peanut stripe virus	Groundnut stripe disease
Phoma pinodella	Leaf spot of pea
Phytoplasma aurantifolia	Lime witches' broom phytoplasma
Podosphaera xanthii	Powdery mildew of cucurbits
Pratylenchus penetrans	Nematode, northern root lesion
Pseudocercospora griseola	Angular bean leaf spot
Pseudomonas savastanoi pv. *phaseolicola*	Halo blight of beans
Pythium debaryanum	Damping-off
Rotylenchulus reniformis	Reniform nematode
Scutellonema bradys	Yam nematode
Scutellonema clathricaudatum	

(continued)

Table 10.5 (continued)

Pest	Common name
Sitophilus oryzae	Lesser grain weevil
Thysanoplusia orichalcea	Slender burnished brass moth
Tribolium castaneum	Red flour beetle
Trichoplusia ni	Cabbage looper
Uromyces appendiculatus	Bean rust
Verticillium dahliae	Verticillium wilt
Xanthomonas axonopodis pv. *phaseoli*	Bean blight

yellow India mosaic virus (MYIMV) (Baker et al. 2013; Markham et al. 1994). Fortunately, as tobacco whitefly is one of the most important crop disease vectors, its occurrence, distribution and transmission mechanisms are well understood, which could potentially help combat the spread of MYMV in mung bean. There is no clear consensus on whether the MYMV resistance genes are monogenic or digenic traits, which in theory could make breeding resistant plants easier; however, variations in pathogen load and other factors could make the breeding process more difficult (Alam et al. 2014).

10.7.3 Fungal Pathogens

Traditionally, fungi are not well-studied. Only recently, owing to rapid improvements in molecular techniques such as polymerase chain reaction (PCR) and NGS, have mycologists been able to grasp the scale of this kingdom and to accurately identify fungal species (Blackwell 2011; Hawksworth and Rossman 1997). This improvement in molecular techniques will indeed lead to better fungal identification and allow for better diagnosis of fungal diseases in mung bean. One of the most important fungal pathogens is *Cercospora canescens* Ellis & G. Martin, which causes the foliar disease known as *Cercospora* leaf spot (CLS) in mung bean and other agriculturally-important plants. *Cercospora canescens* belongs to the Ascomycota phylum, which contains many other important plant and human pathogens. This pathogen also infects other closely-related legume species such as *Vigna unguiculata* (cowpea) and *Phaseolus vulgaris* (common bean) (Dhingra and Asmus 1983; Williams 1975). Even though it can reduce yields by approximately 40%, there is little consensus on the genetic basis for CLS resistance, and whether the resistance gene is monogenic or multigenic is a source of disagreement (Chankaew et al. 2011). What makes breeding CLS-resistant lines or cultivars more difficult is the variation of *C. canescens* among strains, even when isolated from the same region and the same host (mung bean). This pathogen shows variation in terms of

pigmentation and mycelial characteristics, as well as genetic markers such as ITS and RAPD markers (Joshi et al. 2006). Indeed, the natural variation in this species may hinder the development of CLS-resistant lines.

10.7.4 Bacterial Pathogens

Traditionally, isolating and culturing pure microorganisms has been difficult, especially for obligate pathogens, as artificial or natural medium may not be able to replicate the niche of the pathogen due to our limited understanding of a particular organism (Stewart 2012). *Xanthomonas axonopodis* pv. *phaseoli*, previously known as *Xanthomonas phaseoli*, belongs to the class of gammaproteobacteria that causes bacterial leaf blight. Depending on the pathovar, this bacterium can affect a wide range of plant species, from legumes (pv. *phaseoli*) to citrus (pv. *citri*). *Xanthomonas axonopodis* affects a wide range of hosts, including common beans, mung bean, black gram, cowpea and so on. One of the most effective prevention methods for *X. axonopodis* is pretreating seeds since they represent the primary source of this pathogen (Baker and Smith 1966). *Xanthomonas axonopodis* has potentially devastating effects on crop yields and poses one of the major constraints to common bean production in Ethiopia, as it causes common bean blight (Belete and Bastas 2017). However, it is not yet clear whether this is also the case in mung bean.

10.7.5 Nematodes Affecting Mung Bean

Nematodes, belonging to the kingdom Animalia, include various pathogens affecting animals and plants. Although they are often viewed as having harmful impacts on agriculture or human health, some nematodes play important roles in ecology, such as the essential cycling of nutrients and toxins alike (Barker et al. 1994). *Heterodera cajani* Koshy (pigeon pea cyst nematode, also referred to as *Heterodera vigni* Edward & Misra), is a nematode that infects crops such as pigeon pea, mung bean and some *Phaseolus* species. Nematodes can affect both the dry matter content and grain yield of crops. In some cases, up to 86% of grain yield can be lost due to nematodes. Furthermore, to properly control nematodes, a population-monitoring system is required throughout the growth period (Saxena and Reddy 1987). Various methods have been tested for their nematocidal efficiency against *H. cajani*, including using fungi or essential oil derived from herbs (Sangwan et al. 1990; Siddiqui and Mahmood 1996).

10.8 Conclusion and Prospects

As mung bean has become an important crop in many Asian countries due to its high nutritional contents, most studies in mung bean have focused on yield-related traits, such as resistance to yellow mosaic disease (Kitsanachandee et al. 2013), bruchid (Mei et al. 2009), *Cercospora* leaf spot (Chankaew et al. 2011) and other domestication-related traits (Isemura et al. 2012). Since mung bean is mainly cultivated in developing countries, relatively little attention has been paid to this crop, and progress in mung bean breeding has been slow due to the lack of genomic information. In 2014, the reference genome sequence of mung bean was published, allowing breeders and researchers to study the genetic and genomic backgrounds of agronomically-important traits. A number of markers and putative QTLs have been developed based on this genome sequence, along with phenotypic data, represent valuable resources for identifying and locating casual genes for important traits. The use of genomic information from other legume species, especially soybean, will help researchers investigate QTLs and identify candidate genes in mung bean through analysis of sequence similarity and synteny.

A mung bean core collection consisting of 1481 accessions developed by AVRDC-The World Vegetable Center has been used to evaluate various agronomic traits, such as plant height, flowering time, 1000 seed weight and so on (Schafleitner et al. 2015). This collection could be valuable for genome-wide association studies (GWAS) to further detect promising candidate genes.

Collections of wild mung beans from diverse origins grown under different climatic conditions are needed. Analysis of the genetic diversity of these lines would help breeders develop cultivars that can grow in semiarid regions by investigating allelic variations in beneficial traits, especially drought tolerance. As mung bean is a fast-growing crop with a small genome, it represents a good model system for genomic studies of legume species. The recent availability of a mung bean reference genome sequence has facilitated translational genomics studies using the well-studied soybean genome, which could in turn facilitate QTL analysis, GWAS and candidate gene identification in mung bean.

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Appendices

Appendix I: Research Institutes Maintaining Mung Bean Germplasms

Institution	Country	Contact information and website
AVRDC-The world vegetable center	Taiwan	http://avrdc.org
National bureau of plant genetic resources of the Indian council of agricultural research	India	http://www.nbpgr.ernet.in
Institute of crop germplasm resources, Chinese academy of agricultural sciences	China	http://www.cgris.net/cgrisngb.html
Plant genetic resources conservation unit, University of Georgia	USA	https://www.ars.usda.gov/southeast-area/griffin-ga/pgrcu/
University of the Philippines	Philippines	https://www.up.edu.ph/
Rural Development Administration	South Korea	https://www.rda.go.kr

Appendix II: List of Mung Bean Mini Core Collection Provided by AVRDC-The World Vegetable Center

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI000020	4.8	1.9	28	40	53	9	8.8	71.47	Southeast Asia
VI000099	4.1	1.6	22	56	43	8.3	12	32.39	South Asia
VI000105	4.3	1.5	25	42	43	7.2	10.8	35.29	South Asia
VI000164	4.1	1.5	30	34	43	5.6	10.2	29.69	Southwest Asia
VI000170	4.2	1.4	14	33	40	7.5	10	33.71	Southwest Asia
VI000175	4.8	1.8	26	43	43	7.6	10.8	38.67	South Asia
VI000188	4.5	1.5	19	43	40	7.1	11.6	28.88	Southwest Asia
VI000203	4.2	1.5	21	35	43	7.4	11.4	30.98	Southwest Asia
VI000212	4.2	1.6	25	43	43	5.3	9.6	27.34	North America
VI000232	4.9	1.7	20	33	40	8.3	10.6	37.71	Southwest Asia
VI000238	4.4	1.5	21	45	43	6.7	11.4	30.19	Southwest Asia
VI000253	4.2	1.6	19	58	46	8	12.8	31.13	South Asia
VI000316	4.5	1.5	18	34	43	7	9.8	31.21	Southwest Asia
VI000317	4.1	1.4	14	40	43	7.7	12.2	33.87	Southwest Asia
VI000319	4	1.4	17	34	43	6.7	10.8	30.92	Southwest Asia
VI000380	5.8	2.3	23	47	43	9.7	9	70.69	Southeast Asia
VI000461	4.2	1.6	26	54	46	7.8	12.2	32.09	Southeast Asia
VI000470	5	2	19	35	43	8	8.6	66.94	Southwest Asia

(continued)

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI000532	4.4	1.5	19	49	46	7.6	14.4	26.46	South Asia
VI000537	4	1.5	25	47	46	7.1	12.6	25.9	South Asia
VI000542	3.7	1.5	18	28	43	6.5	10.6	25.29	South Asia
VI000551	4.1	1.5	22	41	43	6.7	11.8	28.62	South Asia
VI000554	4.2	1.6	21	43	43	7.1	11.6	30.23	South Asia
VI000559	4.4	1.4	23	43	43	7.1	11.6	29.35	South Asia
VI000578	4.3	1.4	28	63	53	7.1	10.4	26.99	South Asia
VI000589	3.9	1.5	22	39	40	7.1	11.4	33.25	South Asia
VI000616	4.7	1.9	20	50	39	7.6	13.6	32.76	South Asia
VI000618	4	1.5	25	29	40	6.1	8.6	31.83	South Asia
VI000625	3.9	1.6	21	51	43	7.5	11.6	35.66	South Asia
VI000680	5.1	2	23	33	40	7.3	11.4	39.58	North America
VI000723	4.5	1.6	22	29	40	7	10.6	29.85	Southwest Asia
VI000732	4.9	1.9	19	35	43	6.6	9	39.7	South Asia
VI000735	4.6	1.8	21	41	47	6.9	10.6	39.3	South Asia
VI000736	5.4	2	26	60	53	9.9	11.4	60.78	South Asia
VI000749	4.9	1.7	23	51	43	7.7	11	31.98	South Asia
VI000764	4.7	1.7	20	61	43	6.6	10	37.88	South Asia
VI000766	4.4	1.7	23	46	40	7.1	11	34.56	South Asia
VI000805	4.9	2	24	39	43	8.1	11.8	48.38	South Asia
VI000815_1	4	1.5	19	26	53	8.2	9.4	34.74	South Asia
VI000818	5	1.9	22	44	40	7.2	10.6	30.97	South Asia
VI000852	4.3	1.6	17	20	40	7.1	10.8	30.94	South Asia
VI000938	4.3	1.4	16	44	47	7.1	9.8	33.49	South Asia
VI000942	4.4	1.8	17	41	40	8.8	10.8	46.06	South Asia
VI000953	5.1	2.2	24	44	43	8.6	10.8	66.37	South Asia
VI000981	5.1	1.8	20	33	42	7	10.8	38.47	Southeast Asia
VI001023	5.4	2	18	43	43	8.1	12.2	34.71	South Asia
VI001066	4.6	1.9	21	39	53	8.6	11.8	40.68	Oceania/Pacific
VI001096	5.5	2	21	37	43	8	12	52.69	Oceania/Pacific
VI001124	5.6	2.4	28	58	45	7.9	9.6	65.3	Oceania/Pacific
VI001126	4.8	1.8	47	56	53	8.6	13.6	39.77	Oceania/Pacific
VI001162	4.8	1.7	13	47	42	6.4	11	27.34	Oceania/Pacific
VI001191	5	1.9	26	50	46	9.9	13.8	52.93	Southeast Asia
VI001211	5.3	2.1	22	50	46	7.7	9.8	53.68	Southeast Asia
VI001221	5.6	2.2	28	45	44	9.1	10.6	69.72	Southeast Asia
VI001244	5.3	2.4	26	43	44	8.7	7.4	74.78	Southeast Asia
VI001268	5.1	1.8	20	39	46	6.5	10	34.34	South Asia
VI001282	4.9	1.7	21	43	43	7.4	13	31.64	South Asia
VI001284	5	1.7	21	42	43	5.8	8.8	31.7	South Asia
VI001339	5.9	2.3	28	61	46	9.8	10.8	64.42	Southeast Asia
VI001385	4	1.3	27	46	53	6.8	11.4	29.29	South Asia

(continued)

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI001400	4.6	1.6	23	58	46	6.6	10.6	34.27	South Asia
VI001403	4.1	1.6	35	38	53	7.5	13	32.22	South Asia
VI001406_1	3.9	1.5	17	49	43	7	9.8	32.79	South West Asia
VI001408	4	1.5	21	52	43	7.4	11.6	30.18	South Asia
VI001411	4.5	1.5	25	49	43	6.3	10.8	27.38	South Asia
VI001412	4.8	1.8	28	66	47	7.2	11	33.41	South Asia
VI001419	4.4	1.8	21	50	43	6.8	10.6	28.78	South Asia
VI001435	4.6	1.7	18	37	43	6.4	8.2	31.76	North America
VI001448	3.7	1.5	15	58	43	6.9	10.4	21.15	South Asia
VI001471	4.2	1.5	27	43	43	7.1	9.6	33.54	South Asia
VI001482	5.2	1.8	21	45	43	7.8	12.8	31.99	South Asia
VI001490	4.3	1.6	15	43	40	6.6	10.6	29.79	South West Asia
VI001509	4.4	1.6	17	40	43	6.6	12	32.21	South West Asia
VI001514	4.7	1.7	18	48	43	7	11.4	31.03	South Asia
VI001520	4.1	1.4	18	24	40	6.7	11.8	23.85	South Asia
VI001533	4.6	1.6	27	59	53	7.3	12.4	29.89	South Asia
VI001535	4.6	1.7	22	56	45	6.8	11.8	52.82	South Asia
VI001539_1	3.9	1.4	39	71	55	7.6	11	28.62	South Asia
VI001548	4.6	1.7	33	73	53	7.1	11.2	30.45	South Asia
VI001556_1	4.1	1.3	38	72	43	6.6	10	27.98	South Asia
VI001557	4.5	1.6	18	39	47	6	8.4	30.11	North America
VI001562	4.1	1.5	14	36	47	6.9	10.2	29.11	South Asia
VI001576	4.1	1.4	26	52	43	8.6	12.8	28.51	South Asia
VI001579	4.8	1.7	20	48	40	7.1	13	34.95	South Asia
VI001605	4.4	1.7	21	50	43	6.9	11	31.39	South Asia
VI001612	4.7	1.8	22	41	45	6.7	9.4	30.8	unknown
VI001628	4.9	1.8	21	44	43	6.5	11.2	28	South Asia
VI001651	4.2	1.6	18	34	44	6.1	8.6	33.35	South Asia
VI001652	4.3	1.4	17	44	46	6.6	11.6	33	South Asia
VI001654	4.1	1.5	19	48	47	7.1	10.8	31.85	South Asia
VI001678	4.9	1.9	14	23	41	6.7	10.8	36.83	South Asia
VI001692	4.3	1.4	23	29	43	7.1	11.4	27.29	South Asia
VI001698	5.1	2	20	31	42	7.7	13.2	32.92	South Asia
VI001728	4.4	1.7	18	34	43	5.7	10.4	28.18	South Asia
VI001733	5.3	2	18	41	42	6.9	9.2	40.23	South Asia
VI001743	4.3	1.5	14	36	43	6.8	12.2	31.84	South Asia
VI001756	4.6	1.7	19	45	44	7.4	12	34.48	South Asia
VI001762	4.3	1.5	20	48	45	6.6	11.8	31.9	South Asia
VI001806	4.4	1.5	24	42	40	6.3	8.8	28.48	Southwest Asia
VI001806_1	5.6	1.9	20	34	40	8.2	11.4	33.16	Southwest Asia
VI001820	4.2	1.7	20	50	48	7	11	32.08	Europe
VI001859	5.1	1.8	37	46	53	9.4	11	48.89	Southeast Asia

(continued)

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI001974	4.6	1.8	21	33	44	7.2	11.4	36.62	East Asia
VI001993	4.5	1.9	24	42	45	7.7	11.6	37.51	East Asia
VI002009	4.1	1.6	17	25	44	6.9	11	33.63	South Asia
VI002012	4.7	1.8	22	21	46	7.6	8.8	33.21	South Asia
VI002051	4.9	1.7	26	59	46	6.7	10.8	36.36	South Asia
VI002063	5.1	1.9	38	49	46	9.7	13.2	45.67	North America
VI002173	4.6	1.6	20	56	48	7.3	11.4	36.8	South Asia
VI002173_1	4.8	1.8	23	51	48	7.5	12	32	South Asia
VI002176	4.6	1.7	17	37	40	7.7	13.2	31.1	South Asia
VI002176_1	4.4	1.8	18	43	40	7.2	12.2	34.2	South Asia
VI002190	4.3	1.6	19	22	43	6.4	10.6	30.13	South Asia
VI002195	5.4	2.1	29	43	43	8.8	11.2	57.9	Southeast Asia
VI002197	5.3	1.9	20	40	44	7.8	10.6	37.7	East Asia
VI002206	5.2	2.1	27	35	42	8.7	11.8	50.01	Southeast Asia
VI002239	4	1.5	16	25	42	5.2	5.8	34.3	Southwest Asia
VI002274	5.9	2	14	53	43	5.5	6.4	56.2	Southwest Asia
VI002284	3.9	1.5	13	28	41	6.6	11	34.1	Southwest Asia
VI002402	4.6	1.7	24	32	43	8.1	12.6	50.5	Southeast Asia
VI002432	4.8	2	18	27	50	9.1	11	57.4	Southeast Asia
VI002437	4.3	1.6	23	46	46	7.5	12.4	35.6	East Asia
VI002456	5.2	1.7	12	40	43	11.2	13.6	52	East Asia
VI002469	4.4	1.9	23	66	50	8.8	13	51.6	Southeast Asia
VI002487	4.6	1.5	20	51	43	7.5	12.2	31	Southwest Asia
VI002523	5.2	2.2	18	48	60	7.9	11.8	52.5	Southeast Asia
VI002529	5	2	19	63	50	5	7.4	57.1	Southeast Asia
VI002532	4.8	1.9	21	28	44	7.7	8.6	38.2	South Asia
VI002537	4.7	1.9	16	18	44	6.8	10.2	43.3	Southwest Asia
VI002569	5.2	1.8	22	50	50	8.6	11.4	53.5	Africa
VI002587	5.3	1.8	14	26	42	7.8	11.4	54.1	Oceania/Pacific
VI002611	4.5	1.9	19	46	49	8.1	10.4	48.3	Southeast Asia
VI002646	4.5	1.8	19	32	50	7.9	9	56.5	Southeast Asia
VI002647	5.1	2.1	26	36	49	8.9	8.6	69.3	Southeast Asia
VI002672	5	2	22	32	49	8.3	10.4	54.3	Southeast Asia
VI002739	5	1.9	19	24	49	9	9	68.3	Southwest Asia
VI002802	3.7	1.3	17	22	40	6.2	10.4	29.3	Southwest Asia
VI002859	4	1.4	12	29	40	6.5	10.4	33.8	Southwest Asia
VI002860	4	1.5	10	23	39	5.6	8.4	31.3	Southwest Asia
VI002872	4.6	1.7	13	41	41	6.8	12.2	36.2	Southwest Asia
VI002877	5.2	1.8	25	51	44	8.2	12.4	43.9	Southwest Asia
VI002894	4.4	1.7	27	45	49	8.2	13.6	40.4	Southwest Asia
VI002926	4.2	1.5	23	47	45	7.1	11	32.2	South Asia
VI002934	4.6	1.7	30	54	45	7.9	13.4	34.9	South Asia

(continued)

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI002986	4.1	1.3	21	39	47	7	10.6	34.2	South Asia
VI002993	5	1.6	38	66	45	7.5	13	31.1	South Asia
VI002999	4.2	1.5	29	50	45	7.2	11.8	33.1	South Asia
VI003019	4.1	1.7	32	41	43	7.6	11.4	40	unknown
VI003019_1	4.3	1.7	34	49	44	7.3	11.2	42	unknown
VI003034	4.1	1.6	30	48	46	7.4	13.2	30.1	South Asia
VI003035	4.4	1.6	33	56	45	6.8	11.4	31.2	South Asia
VI003057	4	1.4	21	49	45	7.8	11.4	30.9	South Asia
VI003062	4.4	1.6	29	48	45	7.5	10.2	32	South Asia
VI003068	4.2	1.5	25	51	45	7.5	10.8	30.7	South Asia
VI003070	4.6	1.7	30	49	45	7.1	11.8	29.8	South Asia
VI003083	4.4	1.6	27	49	43	7.6	10.8	33.2	South Asia
VI003114	3.8	1.3	31	47	45	6.6	11.6	29.8	South Asia
VI003135	3.9	1.4	34	43	49	6.3	10	24.7	South Asia
VI003159	3.9	1.6	24	66	49	6.5	11.6	26.7	South Asia
VI003172	4.4	1.6	27	52	45	7.1	10.8	33.8	South Asia
VI003181	4.5	1.6	13	30	46	6.8	11.6	36.6	South Asia
VI003183	3.8	1.5	23	51	46	7.9	14.4	36	South Asia
VI003187	4.2	1.8	19	40	46	7.2	12.2	40	South Asia
VI003212	4.1	1.5	13	28	41	6.5	11	32.6	South Asia
VI003220	5.2	1.7	28	52	45	7.3	10.6	42	South Asia
VI003232	4.5	1.8	23	55	47	6.7	12.2	29.7	South Asia
VI003235	4.4	1.7	23	52	48	7.2	11	32.9	South Asia
VI003242	4.7	1.7	25	41	44	7.2	10.4	44.6	South Asia
VI003251	4.8	1.7	25	43	46	6.7	12.2	25.6	South Asia
VI003251_1	4.8	1.8	23	43	46	6.8	11.6	31.4	South Asia
VI003252	4.3	1.5	21	45	46	6.4	12.8	31.7	South Asia
VI003255	4.3	1.7	17	48	48	6.3	11.2	28.6	South Asia
VI003276	5.4	1.6	18	35	48	7.5	13	26.2	South Asia
VI003329	4.5	1.7	30	63	48	7.8	13.4	34.4	South Asia
VI003332	4.7	1.6	27	47	46	6.9	11.6	35.8	South Asia
VI003337	4.5	1.5	28	61	47	7.5	13	33.3	South Asia
VI003364	4.3	1.7	21	37	46	9.1	11.8	50.6	South Asia
VI003379	5.1	1.8	37	60	47	8	12.8	41.9	South Asia
VI003382	4.2	1.6	34	47	48	6.9	11.4	41.2	South Asia
VI003407	3.6	1.3	18	51	48	7.1	9	31.2	South Asia
VI003413_1	4.8	1.6	26	44	45	7.2	11.8	37.5	South Asia
VI003440	4.5	1.6	26	47	45	7	9.8	38.3	South Asia
VI003455	4.7	1.6	23	51	46	7.2	12	39.1	South Asia
VI003456	4.3	1.5	36	52	48	6.5	10	30.3	unknown
VI003465	4.1	1.7	21	48	47	7.4	11.8	33.6	South Asia
VI003470	3.7	1.4	34	52	55	6.9	11	25	South Asia

(continued)

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI003480	4.6	1.8	23	60	47	7.8	10.8	59.9	South Asia
VI003490	4	1.6	19	40	46	6	9.8	31.6	South Asia
VI003493	3.7	1.4	18	39	45	6.6	10.8	31	South Asia
VI003514	4.7	1.5	19	43	44	6.7	10.4	31.1	South Asia
VI003517_1	5.1	1.6	34	58	44	6.7	10.6	29.9	South Asia
VI003534	4.8	1.7	17	44	45	7.2	9	33.8	South Asia
VI003534_1	4.3	1.5	20	56	46	7.8	11.8	37.1	South Asia
VI003548	4.2	1.5	23	53	47	7	11.8	30.6	South Asia
VI003554	4.5	1.5	36	65	49	6.7	10.2	36.1	South Asia
VI003560_1	4.4	1.5	24	55	46	6.3	8.2	32.7	South Asia
VI003563	4.4	1.4	33	65	47	7.1	11.4	36.9	South Asia
VI003577	4.3	1.5	25	50	48	6.8	9.8	34.1	South Asia
VI003602	4.8	1.9	24	57	49	8	10.4	42.1	South Asia
VI003642	4.8	1.5	24	54	46	7.5	12	40.9	South Asia
VI003648	4.5	1.5	30	43	45	7.8	11.6	38.9	South Asia
VI003658	4.3	1.7	27	44	45	7	12	37.8	South Asia
VI003664	4.4	1.6	30	46	46	8.3	12.4	38.8	South Asia
VI003678	4.3	1.5	26	42	46	6.4	11.8	36.8	South Asia
VI003685	4	1.5	29	50	47	7.2	11.2	35.2	South Asia
VI003699	3.6	1.3	15	55	48	5.9	10.8	29.4	South Asia
VI003720	4	1.5	18	37	46	6.9	11.8	28.5	South Asia
VI003725	5.9	2	32	35	45	9.1	11.2	42.2	South Asia
VI003733_1	3.9	1.5	26	49	46	6.5	10.8	32.8	South Asia
VI003734_1	4.8	1.8	26	48	46	7.8	11.6	41.8	South Asia
VI003734_2	4.8	1.8	26	48	46	7.8	11.6	45.8	South Asia
VI003744	4.3	1.8	22	51	50	7.5	12	33.9	South Asia
VI003755	4.7	1.8	22	48	44	7.3	11.6	35.7	South Asia
VI003760	4.4	1.6	25	56	47	7.2	11.8	33.7	South Asia
VI003785	4.2	1.5	25	40	43	8	14.4	36.5	South Asia
VI003795	4.1	1.4	19	47	49	6.9	12.4	40.4	South Asia
VI003801	4.8	1.8	27	41	45	8.1	12.2	48.8	South Asia
VI003882	4.7	1.6	15	35	40	6.6	10	29.03	Southwest Asia
VI003886	4.4	1.5	25	45	43	7.6	10.4	41.47	South Asia
VI003886	4.8	1.8	23	35	43	7.5	9.6	39.04	South Asia
VI003893	4.8	1.7	35	53	53	6.9	11.6	38.08	South Asia
VI003894	4.5	1.6	20	58	43	7.4	12	31.73	South Asia
VI003907	4.2	1.6	19	19	40	7.4	11.8	35.07	Southwest Asia
VI003914	4.6	1.5	23	50	43	6.6	11.4	33.53	South Asia
VI003925	4.5	1.6	19	40	43	7	11.6	26.04	South Asia
VI003927	4.7	1.5	26	53	46	6.5	10.6	30.69	South Asia
VI003929	4.2	1.3	23	35	40	6.1	10.4	27.88	South Asia
VI003942	4.4	1.6	19	32	40	5.7	8.6	31.31	Southwest Asia

(continued)

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI003944	4.6	1.7	17	40	40	7.3	11	41.42	Southwest Asia
VI003947	4.9	2	23	46	40	7.6	11.4	42.72	South Asia
VI003948	4.8	1.9	23	43	40	7.6	12	34.05	South Asia
VI003951–2	4.1	1.6	15	46	40	8.3	14.4	36.95	South Asia
VI003954	4.5	1.7	24	35	40	6.9	11	36.55	South Asia
VI003957	4.5	1.6	28	59	43	6.8	11	32.88	South Asia
VI003958	4.6	1.6	25	41	43	7.5	12	33.92	South Asia
VI003959	4.4	1.6	26	56	43	7.4	11.8	31.55	South Asia
VI004006	4.1	1.4	16	52	43	6.8	13.2	30.76	South Asia
VI004010	4.5	1.5	20	50	43	7.7	10.6	37.28	South Asia
VI004024	5	1.7	19	51	47	8.4	11.4	50.5	Oceania/Pacific
VI004044_1	4.2	1.6	16	38	43	7.1	11.8	30.78	South Asia
VI004045	4.3	1.5	19	40	43	7.6	13.4	27.4	South Asia
VI004048_1	4	1.4	16	33	39	6.6	11.4	25.76	South Asia
VI004069	4.7	1.8	25	50	43	7.7	12.8	38.24	South Asia
VI004096	3.9	1.4	25	53	53	6.9	10.8	30.15	South Asia
VI004096_1	3.7	1.4	30	59	53	7.3	10.8	32.04	South Asia
VI004129	3.7	1.6	19	26	43	5.4	9.4	25.66	unknown
VI004138	4.5	1.5	19	49	40	7.3	11.4	39.96	South Asia
VI004145	4.2	1.5	14	28	40	5.8	10.2	32	Southwest Asia
VI004184	4.8	1.7	28	30	44	7.8	12.6	34.3	Europe
VI004243_2	4.5	1.7	19	24	40	6.6	10.4	37.4	Southwest Asia
VI004244	4.8	1.7	30	24	44	6.2	9.4	34.4	South Asia
VI004297	4.3	1.6	18	25	40	6.1	10.2	37.5	Southwest Asia
VI004302	4.3	1.6	16	25	46	6.3	10.4	38.2	Southwest Asia
VI004307	3.8	1.5	19	23	40	6.4	10.8	37.1	Southwest Asia
VI004312	4.5	1.5	21	27	39	7.2	11.2	38.1	South Asia
VI004347_1	4	1.5	20	22	40	6.1	10.6	36.8	South Asia
VI004351	4.2	1.4	20	18	41	6.5	10	34.3	South Asia
VI004423	4.2	1.5	18	21	40	5.8	9.2	34	Southwest Asia
VI004432	3.8	1.5	20	26	40	6	9	37.1	Southwest Asia
VI004480	4.3	1.6	17	20	41	6.3	9.8	34.4	Southwest Asia
VI004639	4.3	1.5	19	29	39	6.2	8.8	36	Southwest Asia
VI004666	4.3	1.5	15	35	40	7.3	10.8	35.2	Southwest Asia
VI004691	3.7	1.5	19	29	40	6.4	11.2	36.7	Southwest Asia
VI004694	4.5	1.8	19	35	40	6.4	10.2	37.9	Southwest Asia
VI004710	3.4	1.4	19	29	43	6.9	9.8	40.7	Southwest Asia
VI004734	4	1.6	16	20	40	5.3	6.2	38.3	Southwest Asia
VI004743	4.5	1.5	24	59	48	7.1	10	32.5	South Asia
VI004789	4.6	1.5	24	54	47	7.7	11.8	32.7	South Asia
VI004810	4.2	1.3	15	33	46	7.3	10.6	36.8	South Asia
VI004811	4.3	1.4	19	36	47	6.9	11.2	30.4	South Asia

(continued)

AVRDC genebank number	Characteristics[a]								Origin
	PLL	PLW	PHF	PHM	DF	PL	SP	SW	
VI004822	3.7	1.3	22	42	45	7	10.4	29.7	South Asia
VI004838	4.1	1.5	15	48	45	6.6	12.2	33.8	South Asia
VI004842	4.3	1.4	21	47	47	7.4	11.8	34.4	South Asia
VI004853	4.8	1.6	17	46	43	7.1	11.8	34	South Asia
VI004871	3.9	1.4	21	46	48	7	11	36.8	South Asia
VI004877	4	1.4	21	48	48	7.9	12	35.7	South Asia
VI004915	4.3	1.4	16	42	45	6.8	11.4	42.7	South Asia
VI004931	4.4	1.4	21	46	46	7.4	10.4	36.4	Southwest Asia
VI004933	5	1.7	22	60	46	7.9	11.4	36.6	Southwest Asia
VI004934	4.8	1.5	21	56	46	6.8	10.8	36.1	Southwest Asia
VI004937	4.3	1.5	25	59	46	7.3	11	35.4	Southwest Asia
VI004942	5.1	1.8	21	47	46	7.6	11	41.8	Southwest Asia
VI004954	5.2	1.8	25	49	44	7.7	11.8	47.1	Southwest Asia
VI004956	4.6	1.6	19	54	46	7.8	11.6	36.2	Southwest Asia
VI004957	4.6	1.5	21	53	47	7.5	11	35.5	Southwest Asia
VI004958	4.4	1.5	18	49	47	7.8	11.6	41.1	Southwest Asia
VI004965	4.7	1.5	23	46	45	7.6	10.8	32.1	Southwest Asia
VI004968	5	1.7	20	51	45	6.7	10	33.8	Southwest Asia
VI004969	4.6	1.6	23	52	45	6.7	10.6	34.8	Southwest Asia
VI004973	4.3	1.5	21	57	47	7.3	10	30.9	South Asia
VI005022	4.2	1.5	16	57	46	6.2	8.8	33.3	South Asia
VI005024	4.5	2.1	19	78	48	5.6	7.6	48.1	East Asia
VI005030	4.3	1.7	66	90	60	7.9	10.6	36.1	Central America
VI005041	5.8	1.8	20	39	46	8.8	9.8	64.6	unknown
VI005066	4.1	1.4	14	34	46	7.7	10.4	34.9	South Asia
VI014178	3.7	1.3	67	94	64	8.3	11.8	33	Africa

Source: AVRDC-The World Vegetable Center, http://avrdc.org/the-avrdc-the-world-vegetable-center-mungbean-vigna-radiata-core-and-mini-core-collections/

[a]Abbreviations: *PLL* primary leaf length (cm), *PLW* primary leaf width (cm), *PHF* plant height at flowering (cm), *PHM* plant height at maturity (cm), *DF* days to 50% flowering, *PL* pod length (cm), *SP* seeds per pod, *SW* 1000 seed weight (g)

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Chapter 11
Pigeonpea (*Cajanus cajan* L. Millsp.): An Ideal Crop for Sustainable Agriculture

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Abstract Pigeonpea [*Cajanus cajan* (L.) Millsp.] is traditionally cultivated as an annual crop in semi-arid regions of the world. It has a number of characteristics such as diverse maturity time, drought tolerance and natural out-crossing which makes it unique among legumes. These traits not only allow its cultivation in diverse environments and cropping systems, but also permit implementation of different breeding methods. Pigeonpea is a crop of sustainable agriculture and poor crop management, exposure to diseases and pests coupled with unpredictable rains hinder crop improvement activities. However, recently partial out-crossing has been exploited to develop cytoplasmic male-sterility (CMS) based hybrid breeding technology. Thus far, three hybrids have been released for cultivation with yield advantages of 30–50% over standard varieties. Pigeonpea R&D now also enjoys a wealth of genomics resources such as a draft genome sequence, resequencing data, candidate genes and markers associated with key traits. Genomics and breeding efforts are underway to make pigeonpea a more sustainable crop and to unlock the genetic diversity present in germplasm to develop new cultivars rapidly.

Keywords Breeding · Crop improvement · Genomics · Next generation sequencing · Pigeonpea

11.1 Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] belongs to subtribe *Cajaninae*, tribe *Phaseoleae,* subfamily *Papilionoideae* and family *Papilionaceae*. Subtribe *Cajaninae* includes 12 genera (van der Maesen 1986). The genus *Cajanus* contains a total of 32 species. Of these, 13 species are endemic to Australia, 8 in the Indian subcontinent, and 1 in Africa. Cultivated pigeonpea is a short-lived perennial shrub that is traditionally cultivated as an annual crop. Pigeonpea holds an important place in subsistence agriculture in Asia, Africa, Latin America and the Caribbean. It is a

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low-input, drought-tolerant legume with a large temporal variation (90–300 days) for maturity. These unique traits allow its cultivation in diverse environments and cropping systems. Globally, it is cultivated on 7.03 million ha with an annual production of 4.89 million mt and productivity of 695 kg ha^{-1} (FAOSTAT 2017). Around 64% of total production of pigeonpea comes from India; Myanmar and Nepal are the other major pigeonpea growing Asian countries. Kenya, Malawi, Uganda, Mozambique and Tanzania are major pigeonpea-growing countries of Africa.

Pigeonpea was domesticated from its wild ancestor {*Cajanus cajanifolius* (Haines) Maesen} about 3500 years ago (van der Maesen 1990; Varshney et al. 2017a, b; Vavilov 1951). India is the center of origin of pigeonpea and Australia and Africa are important centers of diversity (van der Maesen 1990). Recent evidence generated from whole-genome resequencing (WGRS) of pigeonpea landraces and their wild relatives validate that pigeonpea dispersed from India to Sub-Saharan Africa and finally to South America and Mesoamerica (Varshney et al. 2017a).

Traditionally pigeonpea cultivars are grown in varied intercropping systems involving early maturing cereals and legumes (Saxena et al. 2018a). Sole cropping of pigeonpea also emerged with the development of high-yielding early-maturing cultivars, but it is limited to some specific niches (Saxena et al. 2018a). Pigeonpea seeds are a rich source of protein, carbohydrate, phosphorus, calcium, potassium, crude fiber, fat and vitamins (B1, B3, B9, etc.) important in a vegetarian diet (Saxena et al. 2010a, b, c). Pigeonpea has multiple usage, including food (de-hulled split, green peas), fodder (green leaves, silage), feed (crushed dry seeds) and fuelwood (dry stems). Besides these, being a legume crop, it rejuvenates soil by fixing atmospheric nitrogen (Kumar Rao et al. 1983) and adds organic matter and nutrients through leaf litter. Its deep rooting system also helps to combate short spells of drought, breaks hard soil pan and increases soil water infiltration. Pigeonpea roots are also active in releasing soil-bound phosphorus (Ae et al. 1990). With the above-mentioned advantages and low production costs, pigeonpea has become a preferred crop of poor farmers in sustainable agricultural systems and contributes significantly towards nutritional food security in developing countries. Pigeonpea, in the past, did not receive its due investments towards research and development because of the enhanced emphasis on increasing cereal production through the Green Revolution to tackle widespread global hunger, especially in the Third World.

Genetic enhancement in pigeonpea began in the early twentieth century on a small scale with the evaluation of field collections. This led to the identification of disease-resistant landraces, but did not lead to a significant impact on productivity (Mahata and Dave 1931; Shaw et al. 1933). During the second half of the twentieth century, research organizations such as the Indian Council of Agricultural Research (ICAR), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Indian state agricultural universities initiated pigeonpea improvement programs (Ramanujam and Singh 1981). Under these programs around 100 pigeonpea varieties were released for cultivation. This resulted in a quantum jump in cropped area (+54%) and gross production (+56%). The productivity of the crop, however, remained unchanged with a productivity plateau at around 700 kg/ha. A breakthrough

in pigeonpea productivity was achieved recently when a farmer-friendly hybrid breeding technology was developed at ICRISAT (Saxena et al. 2018b).

The limited genetic diversity in the primary *Cajanus* gene pool and complex nature of abiotic and biotic stresses are considered the major bottlenecks in overcoming the problem of yield stagnation. The evolution of genomics, particularly genomics-assisted breeding (GAB), has now demonstrated its potential to enhance yields in rice, etc. through minimizing losses caused by various biotic and abiotic stresses (Varshney et al. 2005, 2007). GAB in pigeonpea began with the availability of a large number of molecular markers, mapping populations and a draft genome sequence (Varshney et al. 2012, 2017b). The reference genome sequence has been the base of resequencing of >300 pigeonpea lines (Saxena et al. 2017a, b; Singh et al. 2016a, b; Varshney et al. 2017a), which has provided trait-associated markers/genes for important traits and an acceleration of the development of improved pigeonpea varieties. Therefore, it is essential to continue and expand research efforts in pigeonpea improvement, especially through the use of modern genomics approaches for sustaining production.

In this chapter we present an overview and recent updates on different aspects of pigeonpea improvement programs, such as quality germplasm, exploitation of hybrid vigor, application of genomics, wide-hybridization, etc. In addition, strategies using modern breeding platforms to enhance pigeonpea productivity and stability are discussed.

11.2 Adaptation Trends

11.2.1 Pigeonpea Maturity Groups

The primary gene pool of pigeonpea contains large variation in terms of the time needed to mature a crop. The earliest types mature in 85–90 days, while at the other extreme some genotypes take 280–290 days to mature; this huge variation is almost continuous. Saxena et al. (2018b) reported 11 maturity groups established within pigeonpea germplasm. However, for practical purposes, 5 maturity groups are popular and the breeding programs have been established around them (Table 11.1).

Table 11.1 Most preferred maturity groups in pigeonpea and reference varieties

Maturity group	Popular group	Days to flower	Days to maturity	Reference variety/line
0	Super early	45	90	MN4
0	Extra early	65	110	ICPL 88039
I	Early	80	130	UPAS-120
V	Mid early	105	160	Maruti
VI	Medium	120	180	Asha
IX	Late	140	260	Gwalior 3

Source: Saxena et al. (2018b)

These maturity groups are adapted to specific environments and cropping systems and the breeders use the reference cultivars as controls to develop new high-yielding cultivars suitable for their areas.

11.2.2 Adaptation to Latitude

Pigeonpea is predominantly a crop of semi-arid regions and grows between 30° N and S latitudes. Optimal temperatures for crop growth range from 25 to 30 °C, with mean annual rainfall of 600–1400 mm, and daily global solar radiation from 400 to 430 cal cm^{-2}/day. Pigeonpea can be grown in a range of soil types including Entisols, Alfisols and Vertisols, and in soils with pH values of 6.5–8.5 (Reddy and Virmani 1981). The traditional long-duration cultivars cannot be grown beyond 30° N or S, due to their extreme short-day requirement for flowering. This adaptation scenario started changing with the breeding of early-maturing cultivars. Wallis et al. (1981) reported that earliness in pigeonpea was closely related to its photo-insensitivity reaction. Thereafter, a number of photo-insensitive pigeonpea cultivars were bred at ICRISAT. To test their adaptability, these were evaluated at nine locations representing a wide latitudinal range of 7–46° N. Some genotypes such as ICPL 83015, 85030 and 85010 produced over 2 mt/ha of grain even at 46° N (Table 11.2). These genotypes facilitated the introduction of pigeonpea into new niches where it was never grown before.

11.2.3 Adaptation to Cropping Systems

Almost all long- and medium-maturing cultivars are intercropped with a range of cereals, legumes or other crops (Saxena et al. 2018a). Pigeonpea intercropping systems, in most cases, not only facilitate efficient use of resources, but also leads to

Table 11.2 Seed yield (mt/ha) of extra-short-duration pigeonpea lines at different latitudes, 1988–1989

	Latitude (°N)								
Genotypes ICPL	7	9	17	23	29	31	32	34	46
83015	2.32	1.48	2.35	1.75	1.06	1.74	3.73	1.86	2.06
83019	2.21	1.39	1.46	1.43	1	1.36	3.58	1.67	1.76
84023	2.34	1.14	1.42	1.83	1.37	1.87	2.99	2.49	1.59
85010	2.79	1.55	1.59	1.88	1.17	1.25	3.16	2.33	2.15
85030	1.52	1.11	1.21	1.29	0.98	1.16	2.52	0.83	2.46
Mean	2.17	1.27	1.65	1.59	1.16	1.67	3.19	1.7	1.77
CV%	22.8	23.3	14.5	29.2	12.9	4.7	17.1	NA	NA

Source: Saxena et al. (2018c)

more productivity per unit area of land. Besides, it also offers other benefits such reductions in wilt (*Fusarium udum* E.J. Butler) and pod borer {*Helicoverpa armigera* (Hübner)} losses (Bhatnagar and Davies 1978). In most intercrop combinations, pigeonpea remains the primary crop; while in a few, such as cotton and groundnut, it serves as a companion crop. Also, the row ratio of the two component crops varies, depending on soil type, availability of moisture, farmers' choice, etc.

11.3 Production Agronomy

Pigeonpea crop production practices vary according to maturity, cropping system, soil type and environmental conditions. Over time, the cultivars adapted to different situations have been bred and their agronomy researched. A generalized description of cultural practices is given below.

11.3.1 Available Inbred Cultivars

Through the All India Coordinate Pigeonpea Project, Indian Council of Agricultural Research, over 100 pigeonpea cultivars have been released. These include cultivars of different maturity groups, plant types, and seed and pod types. Some of them became highly successful relative to their adoption; on the other hand, a number of cultivars never saw the light of day. Some highly popular cultivars in different maturity groups are given in Table 11.1.

11.3.2 Cultural Practices

Pigeonpea fields need at least one deep ploughing before the commencement of the rainy season to remove weeds and to conserve soil moisture; and this should be followed by 2 or 3 runs of a disc harrow. To avoid plant losses due to waterlogging and to foster proper development of roots and nodules, a well-drained field is necessary; for this a ridge-and-furrow system is recommended. Under rainfed cultivation, pigeonpea is sown with the onset of rains. Under high-input conditions, the extra-early lines are sown in early June in northern India; this permits timely sowing of a subsequent wheat crop; while at lower latitudes the planting can be delayed by a fortnight. Optimal seeding depth for pigeonpea is 4–5 cm. In pigeonpea, late sowings invariably result in the reduction of biomass and seed yield. This is due to the sensitivity of plants to short photoperiods which induce flowering before achieving sufficient biomass. Kaul and Sekhon (1975) concluded that grain yield and yield-attributing characters were influenced by date of sowing. They recorded significant reductions in seed yield, plant height, pods per plant and dry matter production, but high harvest indices.

Traditionally, pigeonpea farmers broadcast a seed mixture of the two intercrops on flat beds; but now the ridge-and-furrow system is also becoming popular for the medium- and late-maturity groups. In contrast, sole cropping of early types is always sown in rows. Experiments at the Indian Agricultural Research Institute have shown that pigeonpea sown on a ridge-and-furrow system in the fields prone to waterlogging gave 30% more yield compared to flat sowing (IARI 1971). Plant spacing depends on maturity duration and cropping system. Extra-early pigeonpeas are grown at 30 cm rows with about 10 cm spacing between the plants. In traditional production systems, the plant density could be around 5 plants/m^2 (Pathak 1970). To the contrary, Natarajan and Willey (1980) found that 10 plants/m^2 gave a higher yield than 5 plants/m^2.

11.3.3 Insect Management

Insect management is the most critical operation in pigeonpea cultivation, in all the maturity groups. In most countries, the present-day plant protection systems are dominated by chemicals. Over a period of more than five decades, chemical pesticides use increased by 170 fold; from 2.2 g/ha of active ingredient in 1950 (Handa 1995) to 381 g/ha in 2007. To control pests, pigeonpea farmers usually make 5–8 chemical applications per crop. Repeated use of some insecticide(s) may lead to the development of inherent resistances in the insects against these insecticides. An important aspect of an integrated pest management (IPM) program is to minimize the damage through some designated cultural practices. Ideally, IPM technology involves components such as use of tolerant genotypes, enhancing natural enemies, improving cultural control and judicious use of chemical pesticides.

For pod borer (*Helicoverpa armigera*) control in pigeonpea, the age-old field practice of shaking larvae-laden plants has been found to be very effective and economical. This practice involves manual shaking, collection, removal and destruction of the borer larvae at the flowering/podding stage from their feeding sites. Shaking the plant can dislodge up to 97% of caterpillars of all sizes; the dislodged larvae are then destroyed.

With increased awareness about the harmful effects of pesticides in recent years, the importance of natural enemies to control insects has attracted attention. There are a number of natural enemies of pigeonpea pests; these include (i) predators – those which hunt and consume all or part of their prey (several species of spiders, lizards and birds feed on *Helicoverpa armigera.)*; (ii) parasitoids – those who live on or inside the body of the insect-host, and more than 75 insect parasitoids (Orders: *Hymenoptera*, *Diptera*) attack different growth stages of *H. armigera*; (iii) pathogens – those which infect the crop pests to kill or render them infertile (nematodes, fungi, bacteria, and viruses in nature, which have the potential to kill *Helicoverpa* pod borers). At present, a number of safe and non-target, bio-rational pesticides are also commercially available. Among these, neem (*Azadirachta indica* A.Juss), *Bacillus thuringiensis* Berliner and nuclear polyhedrosis virus (NPV) are popular for protecting the crop.

11.4 Key Breeding Approaches

11.4.1 Pure Line Breeding

In most self-pollinated crops pedigree breeding has been practiced for decades to develop high-yielding cultivars and to incorporate certain special traits. Despite the considerable extent of natural cross-pollination in pigeonpea, breeders have used pure line breeding to develop new cultivars. Through this breeding approach, about 100 cultivars have been bred in India alone, and they have helped to increase the cropped area and production, but not yield. Swaminathan (1973) attributed this failure to low selection efficiency and various inherent physiological and management limitations of the crop. Chauhan et al. (1994), on the other hand, considered that low yield levels of pigeonpea were due to poor partitioning of carbohydrates and the accumulation of huge food reserves in non-reproductive parts of the plant resulting in a low harvest index. The issue of a low-harvest index in pigeonpea has been highlighted by many plant breeders and physiologists, but so for no significant progress has been made to improve this parameter. Perhaps the development of annual types (Saxena et al. 1992) in the future may help to improve the harvest indices in pigeonpea.

11.4.2 Interspecific Hybridization

Genetic divergence plays a key role in crop enhancement programs. It is likely to yield new recombinants when diverse lines are used in hybridization schemes. In pigeonpea, gene banks at ICRISAT (13,632 accessions), NBPGR (11,229 accessions), USDA (4116 accessions) and Kenya (1288 accessions) hold huge germplasm of primary, secondary and tertiary gene pools (Singh et al. 2013; Upadhyaya et al. 2007). These resources represent a wide range of genetic diversity for various morphological and ecological traits (Remanandan 1981). In addition, the wild relatives of pigeonpea are known to carry genes for certain unique traits such as high protein, resistance to diseases and insects, flowering, photoperiod insensitivity, rapid seedling growth, salinity and drought tolerance, etc. (Mallikarjuna et al. 2006; Subbarao 1988). At ICRISAT, a successful search for disease and insect resistance has been made among the wild relatives of pigeonpea. These include *Cajanus scarabaeoides* (L.) Thouars and *C. sericeus* (Baker) Maesen for sterility mosaic and *C. platycarpus* (Benth.) Maesen for phytophthora blight and cyst nematode. Similarly, *C. scarabaeoides* and *C platycarpus* have genes for resistance to pod borers and phytophthora blight. Some of the wild relatives of pigeonpea have yielded CMS systems as well. The molecular analyses undertaken by Yang et al. (2006), Kassa et al. (2012), Saxena et al. (2014) and Varshney et al. (2017a) have also confirmed the presence of vast genetic diversity among wild relatives of pigeonpea. These genetic resources offer immense opportunities for breeders to enrich their breeding

programs and develop desirable cultivars. The use of the primary gene pool is straightforward, but the utilization of wild species is difficult and needs more resources and therefore should be restricted to cases where the resistance in the cultivated types is lacking. Recently, breeders have been utilizing the concept of pre-breeding and genomics information in exploit this gene pool in crop improvement.

11.4.3 Mutation Breeding

Thus far, only five varieties have been bred through mutagenesis. For EMS treatment, 0.6% strength was used to develop cultivar Co 3. Another pigeonpea variety, Co 5, was developed using 16 Kr of gamma rays. Two varieties, TT 5 and TT 6, were developed using fast neutron treatment. Besides high yield, TT 6 exhibited 25% larger seeds than parental line T 21 (Pawar et al. 1991). Variety TAT 10 was developed by mating two mutant pure lines derived from fast neutron treatment.

11.4.4 Population Breeding

Khan (1973) advocated the formation of composites to maintain genetic variability, recombination breeding and selection of single plants for conventional breeding. He also emphasized that after 3–4 generations of random mating these composites can be improved by a suitable system to provide a heterogeneous population, which can be released, to the farmers as an open-pollinated variety. Onim (1981) tested two population improvement methods – stratified mass selection and mass selection with progeny testing – in marginal rainfall areas. He reported 2 and 4% yield gain per cycle under stratified mass selection and mass selection with progeny testing, respectively.

11.5 Breeding Achievements

11.5.1 Disease Resistant Cultivars

Information on the genetic control of pigeonpea diseases is restricted to a limited number of studies. Shaw (1936) reported the presence of two complimentary genes for fusarium wilt (FW) resistance. Joshi (1957) and Pawar and Mayee (1986) concluded that a single dominant gene determined resistance to FW. Saxena et al. (2012) reported that FW resistance in pigeonpea was controlled by one dominant and one recessive gene. Resistance to sterility mosaic disease was reported to be controlled by four independent loci, consisting of two duplicate dominant genes and

two duplicate recessive genes (Singh et al. 1983). Sharma et al. (1984) reported that two genes govern the resistance to sterility mosaic disease (SMD) with multiple alleles for resistance. Srinivas et al. (1997) found two non-allelic recessive genes governed the resistance to race-1 and for race-2, a single gene with three alleles controlled the resistance. For the P2 race of *Phytophthora* blight, resistance was found to be governed by a single dominant gene (Sharma et al. 1982), while a single recessive gene controlled resistance to *Alternaria* blight (Sharma et al. 1987).

Breeders have achieved considerable success in developing high-yielding, disease-resistant cultivars using pure line breeding for FW and SMD. These achievements can be attributed to the development of an effective field screening technique and availability of stable sources of resistance (Nene et al. 1981). Since both diseases cause economic losses in the same ecosystems, a single screening nursery was created, which allowed simultaneous screening for both diseases. In this context, pedigree selection within landraces has been very effective both in India and Africa. For example, variety Maruti, a selection from ICP 8863 collected from Maharashtra State, is proving a boon to the farmers of FW-prone areas of central India (Bantilan and Joshi 1996). Similarly, in Africa, the variety Nandolo wa nswana, a selection from a Tanzanian landrace (ICP 9145), is very popular (Silim 2001).

For *Phytophthora* blight, however, success has proved elusive, due to factors such as the presence of a number of pathogenic races and the difficulty in finding stable resistance sources. *Alternaria* leaf spot (*Alternaria tennissima*) is a disease of late-sown crops and in certain agroecological areas with high humidity. Only two resistant lines ICPL 366 and DA 2 were bred using pure line breeding. Onim and Rubaihayo (1976) bred cultivars UC 796/1 and UC 2113/1 with resistance to *Cercospora* leaf spot in Uganda. Cultivar ICP 9177 was identified as resistant to powdery mildew in Kenya (Raju 1988).

11.5.2 Insect Resistant Cultivars

Given the absence of reliable genetic resistance resources, *Helicoverpa armigera* can cause total losses to the crop all over the world. Research towards identifying reliable host-plant resistance for *H. armigera* pod borer have not yielded reliable results. From a screening of over 10,000 germplasm accessions, not a single stable truly-resistant genotype could be identified. However, a few accessions with relatively less pod damage (tolerant) were identified; and these, however, were inconsistent in the expression of resistance level across seasons and locations. Tolerance to this pest was conditioned by non-preference in ovi-position. Furthermore, a huge intra-accession variability for compensative reproductive regrowth following pest damage further adds to its low heritability. *Maruca vitrata* (Geyer) is another pod borer causing serious damages to the crop. Saxena et al. (2002) reported the selection of some early-maturing germplasm which showed significant yield advantage over controls under non-sprayed conditions. The tolerance to *Maruca* damage was conditioned through yield compensation mechanisms. Poor larval growth and/or

interference in the normal growth cycle of larvae feeding on the tolerant lines were the possible reasons (Sharma et al. 1999). Scientists have not given up hope and are still trying to develop technologies to overcome this menace. Present attempts include the use of wild species and incorporation of alien genes through transformations. Results have shown that some morphological traits in the wild species, such as thick pod wall, prominent pod constrictions and the presence of *C* trichomes on the pod surface, are related to pod borer tolerance in pigeonpea.

In breeding resistant cultivars, the major hurdle is the non-availability of reliable sources of resistance to this pest. Therefore, use of systemic pesticides and integrated pest management techniques are recommended (Saxena et al. 2018c).

11.5.3 Waterlogging Tolerant Cultivars

Temporary waterlogging poses a serious threat to pigeonpea productivity, especially in deep Vertisols. Under waterlogged situations, the anaerobic bacteria become active which leads to a shortage of oxygen in the soil, and adversely affects the growth and development of the submerged pigeonpea crop. A few waterlogging-tolerant pigeonpea genotypes have been identified at ICRISAT (Sultana et al. 2013) which can be used in breeding as donor parents. The tolerant genotypes develop lenticels which help in oxygen intake. The tolerant reaction is known to be controlled by a single dominant gene. Availability of tolerant genotypes, good screening technology and a favorable genetic system provide opportunities to breed high yield waterlogging tolerant cultivars.

11.5.4 Cultivars for Intercropping

The diversity in cropping systems is one of the main factors discouraging pigeonpea breeders to undertake any targeted program. The selection of the crops, cultivar, their maturity, planting ratios, time of sowing and growing environments are but a few variables that produce a range of interactions (Allard 1999), adding to inefficiencies in breeding. Furthermore, even the outcomes achieved, despite the above constraints, could be specific to location, soil type and prevailing climate in each season (Francis 1985). Thus, pigeonpeas have continued to be cultivated on marginal land, mostly under rainfed situations where the risk of crop failure is high. To have more assurance against crop failures and to harvest more food in time and space, most farmers grow pigeonpea as an intercrop with short-cycle cereals and other crops. Presently, intercropping systems account for over 70% of the pigeonpea area. However, the yield of pigeonpea in this system is very low (400–500 kg/ha). The non-availability of improved cultivars adapted specifically to intercropping environments is perhaps the major constraint accounting for low yield. Considering the food and nutritional needs of an ever-increasing human population, productivity enhancement of this high-protein pulse is highly indispensable.

In most rainfed areas pigeonpea is intercropped predominantly with relatively earlier-maturing and fast-growing cereal crops. Until now, there is no well-directed research to define conclusively the constituent components of an ideal pigeonpea plant type that would perform efficiently under different growing conditions. However, by putting the published reports together, it can be inferred that for pigeonpea-cereal intercropping, a pigeonpea cultivar should have non-determinate growth, spreading or semi-spreading branches, a greater number of secondary and tertiary branches, rapid seedling growth, long fruiting branches, more bunches of flowers, 5–6 pods/bunch, 4–6 seeds/pod, 12–14 g/100 seed weight, resistance to wilt and sterility mosaic, deep root system/drought tolerance and the ability to recover from losses (Saxena et al. 2018a).

11.5.5 High-Protein Cultivars

Sufficient quantities of protein in the human diet are essential for normal growth and development. Paucity of this vital growth component among people living at subsistence levels is common and leads to severe malnutrition, particularly among children and women. Animal protein is becoming dearer, day-by-day, and the availability of homegrown vegetable protein is insufficient to meet the domestic needs due to small-holdings, low productivity and the high cost of inputs. This problem is now increasing beyond dangerous proportions, especially in the underdeveloped and developing countries. The per capita protein availability in India, in the decade ending in 2009, recorded a significant decline from the recommended 52 g per person per day to <25 g. To overcome this shortage, there is a need to produce more protein per unit of land area. This is possible either by enhancing yield or by breeding high-protein cultivars. In this respect, pigeonpea stands above most pulses due to its tolerance to various stresses and production of protein-rich (20–22%) grains. At ICRISAT, breeding for high-protein pigeonpea was undertaken and new breeding lines recorded protein content between 28% and 30%, at no loss of productivity. Saxena and Sawargaonkar (2015) reported that such lines yielded additional protein at 100,000 g/ha.

11.5.6 Cultivars for Special Niches

Sole cropping of pigeonpea is of relatively recent origin and emerged with the development of high-yielding, early-maturing cultivars. It is limited to certain specific niches (Saxena et al. 2018a, b, c, d, e) and occupies only 10% of the entire pigeonpea area. The early-maturing cultivars, when sown as a sole crop, exhibit high (34%) harvest indices as compared to 24% for medium-maturing genotypes (Sheldrake and Narayanan 1979). Therefore, from the cropping systems point of view, early-maturing pigeonpea genotypes are preferred by farmers because they provide opportunities to accommodate a follow-up crop, such as wheat, in the same

field; the legume-cereal rotation benefits both crop as well as the soil, besides bringing high economic returns (Dahiya et al. 1977). The recently developed super-early pigeonpea, which matures in about 90 days, are photoinsensitive and can be grown successfully in various new niches (Vales et al. 2012).

11.6 Hybrid Breeding

Pigeonpea yields have remained unacceptably low over recent decades and efforts to break through this undesirable plateau by breeding pure line cultivars has not succeeded. Breeders therefore are looking for an alternative tool to overcome this constraint. Success has come from ICRISAT in the form hybrid breeding technology. In this context the breeding of a commercially-viable cytoplasmic nuclear male sterility (CMS) system using the cytoplasm of *Cajanus cajanifolius* – a wild relative of pigeonpea, was the major breakthrough (Saxena et al. 2005, 2018b, c). Later, a hybrid technology was developed by identifying its fertility restorers.

After extensive testing of hybrid technology across India, the world's first commercial pigeonpea hybrid was released in Madhya Pradesh, jointly by ICRISAT and RVRS VV Gwalior (Saxena et al. 2013). This hybrid has demonstrated 40–50% yield advantage over the best locally-adapted varieties in different agroecological environments, giving a strong indication of a breakthrough in the stagnant productivity of the crop. Soon two more hybrids were released in India (Saxena et al. 2018b). They also reported that besides high yields, the pigeonpea hybrids have additional advantages over the inbred cultivars which can play a great role in enhancing both the productivity and sustainability of the crop. These include rapid seed germination and seedling growth, >30% greater biomass both above and underground, need of 30% less seeding rate than pure line varieties and extra resilience to counter various types of stress.

In All India Coordinated Trials, hybrid ICPH 2671 (2564 kg/ha) was 31% superior to the control. Subsequently, in 1829 on-farm trials, conducted in Maharashtra (782 trials), Andhra Pradesh (399), Madhya Pradesh (360) and Jharkhand (288 trials) states, ICPH 2671 recorded 30–60% superiority over the best local cultivar. Generally, in all four states, ICPH 2671 was 51% better than the control in its productivity (Fig. 11.1). These data clearly demonstrated that in pigeonpea significantly higher productivity levels can be achieved by farmers and the persistent yield plateau can be surpassed. Hybrid seed production is the key factor in the adoption of hybrids. Experiments conducted at ICRISAT have shown that hybrid seed (A × R) yields were recorded at many places in the states of Andhra Pradesh, Madhya Pradesh, Gujarat and Maharashtra. They also documented that on average 1019 kg/ha of hybrid seed yield was possible with ease.

The release of ICPH 2671 was followed by ICPH 2740 and ICPH 3762. All the hybrids are free of wilt and sterility mosaic diseases. The successful development of hybrid technology has provided opportunities to pigeonpea breeders to break the yield plateau.

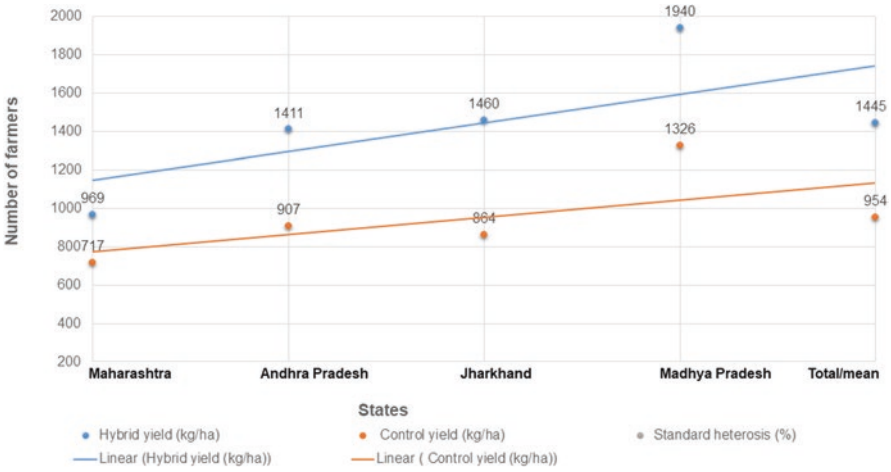

Fig. 11.1 Yield and standard heterosis of pigeonpea hybrid ICPH 2740 and popular cultivar Asha in four states in India, 2009–2011

11.7 Seed Production Technology

Seed production technology must be simple and producer-friendly. The seed production package should contain appropriate agronomic practices including insect management options and postharvest seed handling. Unlike most pulses, the maintenance of seed quality in pigeonpea is rather difficult and resource-intensive due to the considerable extent (20–50%) of natural outcrossing and therefore a safe isolation distance is essential to produce genetically-pure seed. Ariyanayagam (1976), recommended a minimum isolation distance of 180 m and a maximum of 360 m, while Agarwal (1980) recommended distances of 400 m and 200 m for the production of foundation and certified pigeonpea seeds, respectively. The experience at ICRISAT suggested that the isolation of 500 m is safe for the maintenance seed of purity. The seed technology for both pure lines and hybrid cultivars in pigeonpea has now been perfected (Saxena 2006). The results of seed production at ICRISAT have shown that in pigeonpea a seed-to-seed ratio of 1:200 to 1: 300 can easily be obtained.

In a three-line hybrid seed production system, the hybrid seed produced by crossing A- line with R-line, is reported as certified seed and it is grown at a larger scale. For the production of certified seed of hybrids, the A-line and its pollen parent (R-line) are grown in 4:1 ratio in an isolated block. The pollinating insects actively visit the male and female flowers in a random fashion and in the process they collect pollen from fertile plants and carry out hybridization on the male-sterile plants at ICRISAT. From a small (120 m^2) isolation a total of 15 kg seed at 1250 kg/ha of hybrid ICPH 2671 was produced with a row ratio of 1 male: 4 females. Details of the seed production technology are available in the monograph by Saxena (2006).

11.8 Molecular Breeding

In terms of modern crop improvement, a number of success stories are noteworthy in cereals, starting from the Green Revolution in the late 1960s to modern molecular breeding products such as the Swarna-Sub1 flood-resistant rice cultivar (Septiningsih et al. 2009) in the early twenty-first century. Realizing the importance of pigeonpea, a few concerted efforts were initiated in 2005 and reasonable progress has been made. During the last decade, pigeonpea genomics has focused on the development of resources such as molecular markers, genetic maps, transcriptomes, etc. These genomics resources have been used to enhance understanding of genetic control of various economically-important traits and created a base to deploy molecular breeding (Pazhamala et al. 2015). Moreover, the advances in next-generation sequencing (NGS) have helped to fill the need for information on genes at the whole genome level through a draft genome (Varshney et al. 2012). Besides decoding the draft genome, several germplasm lines have also been resequenced (Kumar et al. 2016; Varshney et al. 2017a). Equipped with the draft genome and sequence information on elite lines and germplasm lines, pigeonpea researchers have initiated its deployment in crop improvement programs. As a first step to deploy a marker-assisted backcrossing (MABC) approach, a number of markers associated with desirable traits have been identified (Fig. 11.2).

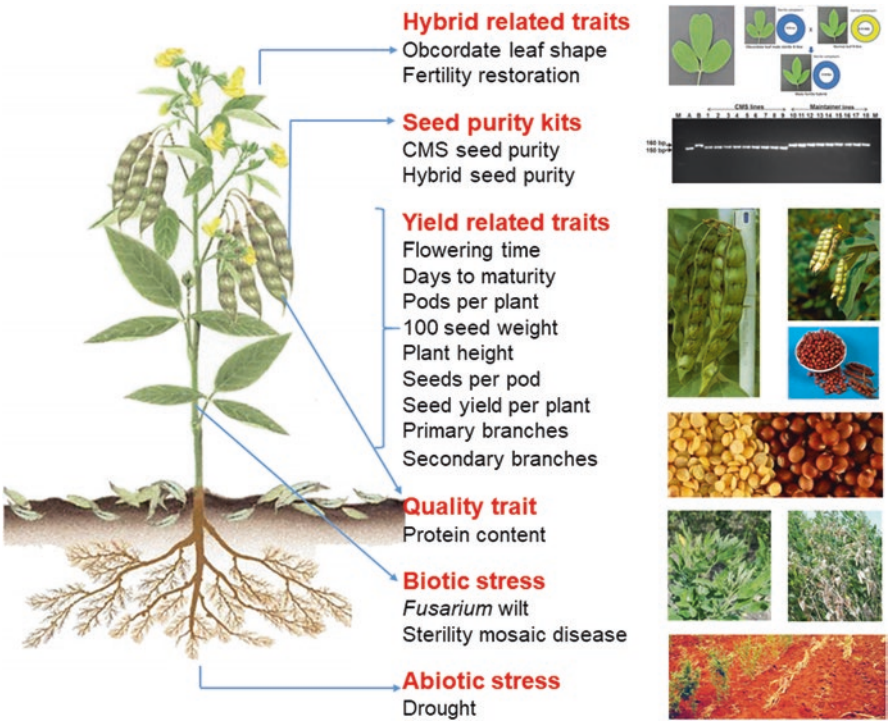

Fig. 11.2 Traits under investigation/or mapped through genomics approaches in pigeonpea

For instance, markers associated with FW resistance (Saxena et al. 2017a), SMD resistance (Saxena et al. 2017b), fertility restoration (Bohra et al. 2012; Saxena et al. 2018a, b, c, d, e), growth habit (Saxena et al. 2017c), different agronomics traits related to plant type and earliness (Varshney et al. 2017a), cytoplasmic male-sterility (CMS) (Sinha et al. 2015), 100-seed weight (Varshney et al. 2017a) and hybrid purity assessment (Bohra et al. 2011; Saxena et al. 2010a, b, c) have been identified. Other traits which are being explored for associated markers include seed-protein content, yield-related traits, water-use efficiency, waterlogging and photoperiod insensitivity, etc. (Fig. 11.2). With the advantage of the abovementioned traits, initial efforts using associated markers are taking place for transferring FW resistance, SMD resistance and earliness in already existing high-yielding varieties. Furthermore, by using genomic segment introgression from wild species (e.g. *C. cajanifolius, C. acutifolius* (F.Muell.) Maesen, *C. scarabaeoides*), a few lines with enhanced resistance to SMD, FW and higher yield have been developed in pigeonpea. These lines are under multilocational trials under AICRP-Pigeonpea in India for possible varietal release.

In order to strengthen the hybrid technology in pigeonpea, a number of genomics approaches are being deployed (Saxena et al. 2015). In this context seed purity holds the key; any level of contamination will lead to the deterioration in hybrid performance with respect to yield and resistance to various stresses. To ensure the quality of hybrid seeds, simple sequence repeats (SSRs)-based hybrid purity testing kits have been developed for two hybrids namely, ICPH 2438 and ICPH 2671 (Bohra et al. 2011; Saxena et al. 2010a, b, c). Additionally, markers for differentiating cytoplasmic male-sterile lines from maintainer lines (Sinha et al. 2015) and fertility restorer (R-) lines from non-restorer (A- and B-) lines (Saxena et al. 2018a, b, c, d, e) in A4 system have been developed. The abovementioned markers are being routinely used for purity assessment in hybrids, as well as in selection of parental lines.

11.9 Conclusions and Prospects

Pigeonpea is a primary source of digestible protein to more than a billion people living across the globe. It is a sustainable crop of resource-poor farmers in the tropics and subtropics. Due to lower production cost, the crop has survived for ages under rainfed farming systems. Moreover, in recent years, global attention is being shifted from *food security* to *nutritional food security* to alleviate malnutrition. As animal protein is unaffordable to masses of people, a pulse crop like pigeonpea has gained importance. Furthermore, the ability of this crop to produce reasonable amounts of seeds, even under adverse growing conditions, enhances its importance as a climate-resilient crop. Substantial progress has been made over the years through systematic crop improvement programs in overcoming the constraints of major diseases like FW and SMD, reducing cropping duration, etc. These efforts have resulted in enhanced production and cultivation area, but not in yield. Since

there is limited scope for horizontal expansion, research efforts should move to achieve yield enhancement. In this direction hybrid technology seems to be a viable option. Simultaneous deployment of modern genomics information in pigeonpea improvement programs is also providing an opportunity to realize enhanced genetic gains. We anticipate that public and private sectors will support pigeonpea improvement programs as they have done in the past for staple grain crops. This will help to enhance the livelihood of the underprivileged.

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Appendix I: Research Institutes Relevant to Pigeonpea

Institution	Specialization and research activities	Contact information and website
Birsa Agricultural University	Plant Breeding and other crop improvement activities	Ramgarh- 834006, Jharkhand http://www.bauranchi.org/
Chaudhary Charan Singh Haryana Agricultural University	Plant Breeding and other crop improvement activities	Hisar- 125004, Haryana http://hau.ac.in/index.php
College of Agriculture, Kalaburgi, UAS, Raichur	Plant Breeding, Entomology, Pathology	Aland Road, Kalaburgi-585102, Karnataka http://www.uasraichur.edu.in/index.php/en/education/colleges/college-of-agriculture-kalaburgi
Dr. Rajendra Prasad Central Agriculture University	Plant Breeding and other crop improvement activities	Samastipur- 848125, Pusa, Bihar https://www.rpcau.ac.in/
ICAR- Indian Institute of Pulses Research	Plant Breeding, Entomology, Pathology, etc.	Kanpur- 208024, Uttar Pradesh http://iipr.res.in/
ICAR-Central Island Agricultural Research Institute	Plant Breeding and other crop improvement activities	Port Blair- 744101, Andaman and Nicobar Islands https://ciari.icar.gov.in/
ICAR-Indian Agricultural Research Institute	Plant Breeding and other crop improvement activities	New Delhi- 110012, Delhi; http://www.iari.res.in/
ICAR-National Bureau of Plant Genetic Resources	Genetic Resources	New Delhi- 110012, Delhi www.nbpgr.ernet.in/

(continued)

Institution	Specialization and research activities	Contact information and website
Indira Gandhi Krishi Vishwavidyalaya	Plant Breeding other crop improvement activities	Krishak Nagar- 492012, Raipur, Chhattisgarh http://www.igau.edu.in/
International Crops Research Institute for the Semi-Arid Tropics	Plant Breeding, Genomics, Entomology, Pathology, Microbiology, Genetic Resources etc.	Hyderabad-502324; Telangana http://www.icrisat.org/
Mahatma Phule Krishi Vidyapeeth	Plant Breeding other crop improvement activities	Rahuri- 413 722, Maharashtra http://mpkv.ac.in/
National Pulse Research Centre	Plant Breeding other crop improvement activities	Puddukkottai- 622303; Tamilnadu
Punjab Rao Krishi Vidyapeeth	Plant Breeding other crop improvement activities	Akola- 444104, Maharashtra http://www.pdkv.ac.in/
R.A.K. College of agriculture Sehore	Plant Breeding other crop improvement activities	Sehore- 466001, Madhya Pradesh
Regional Agricultural Research Station	Plant Breeding other crop improvement activities	Tirupati- 517 506, Andhra Pradesh
Regional Agricultural Research Station	Plant Breeding other crop improvement activities	Warangal- 506007; Telangana
Regional Agriculture Research Station, Lam	Plant Breeding other crop improvement activities	Guntur-522034, Andhra Pradesh
Sardarkrushinagar Dantiwada Agricultural University	Plant Breeding other crop improvement activities	Satsan- 385506, Gujarat http://sdau.edu.in/
VNMKV Agricultural Research Station, Badnapur	Plant Breeding other crop improvement activities	Jalana- 431202, Maharashtra http://www.vnmkv.ac.in/
Zonal Agriculture Research Station	Plant Breeding other crop improvement activities	Khargone- 451001, Madhya Pradesh

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Chapter 12
Soybean [*Glycine max* (L.) Merr.] Breeding: History, Improvement, Production and Future Opportunities

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Abstract Soybean, *Glycine max* (L.) Merr., has been grown as a forage and as an important protein and oil crop for thousands of years. Domestication, breeding improvements and enhanced cropping systems have made soybeans the most cultivated and utilized oilseed crop globally. Soybeans provide a high-quality protein source for livestock and aquaculture, oil for industrial uses and a valued component

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of human diets. Originating in China and Eastern Asia, today 80–85% of the world's soybeans, approximately 88 million ha, are grown in the Western Hemisphere. United States soybean breeding and development efforts for over 80 years have transitioned from primarily universities and United States Department of Agriculture (USDA) programs to private company-led investments in commercial cultivar development. Soybean breeders continuously adapt tools and technologies that encompass classical breeding, mutation breeding and marker-assisted selection, biotechnology and transgenic approaches, gene silencing, and genome editing. In addition to breeding technologies, improved agronomics, precision agriculture and digital agriculture have advanced soybean production and profitability. The primary goals of soybean breeding and cropping systems advances include yield improvement, increased seed protein and oil composition and quality, and yield preservation through weed, pathogen, insect pest and abiotic stress resistance and management. This chapter primarily describes the introduction and improvement of soybeans in the United States. Contributing authors describe classical and molecular breeding, biotechnology, biotic and abiotic stress management, and soybean agronomics and cropping systems improvements that maximize soybean productivity, profitability and sustainability to supply a continually increasing world demand for protein and oil for feed, fuel and food.

Keywords Biotechnology · Cropping systems · Digital agriculture · Gene editing · Genetic diversity · Genomics · Mutation · Operations research

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12.1 Introduction

Soybean, *Glycine max* (L.) Merr., is a leguminous plant in the family Fabaceae (Leguminosae) that originated in China over 3000 years ago. Introduced to North America during colonial times, improvement of soybean beyond a forage crop did not begin until the early 1900s as breeders and growers first attempted to exploit soybean for its high-quality protein and oil. Following WWII, soybean breeding and agronomic research efforts accelerated to improve the plant as an important oilseed row crop. Early soybean breeding focused on changing the plant architecture from a viny morphology to a bushy, upright plant with indeterminate or determinate flowering and pod set with larger and higher quality seed. Breeders developed maturity groups adapted to latitude gradients and growing conditions from northern to southern regions of the United States (US). Photoperiod and average growing season temperatures are the primary determinants for soybean maturity group classification. There are seven primary maturity group zones for US soybeans, beginning with maturity group 0 in the far north (North Dakota) and ending with group VI (6) in the far south (Georgia) (Mourtzinis and Conley 2017).

Today, soybeans are grown in China and other Asian countries, Europe, South America and in the US. Large-scale commercial soybean production is predominantly in the Western Hemisphere with over 33% of the world's supply produced in the US, 32% in Brazil and 17% in Argentina (USDA-FAS 2018a, b). As a row crop, US grown soybean is an economically important and extensively exported cash crop and contributes to agricultural sustainability (Fig. 12.1).

Fig. 12.1 Typical commercial soybean production in the US. (Photo courtesy of Joseph Murphy, Iowa Soybean Association)

Soybeans can improve soil health by providing available nitrogen through their symbiotic relationship with nitrogen-fixing *Bradyrhizobium japonicum* bacteria. Crop rotations that include soybeans facilitate better fertility and disease, insect and weed management strategies for rotated crops. Soybeans are valued primarily for the protein-rich meal that is fed to livestock, especially monogastrics like poultry and swine. Soybean protein is, however, deficient in sulfur-containing essential amino acids and contains trypsin inhibitors and other antinutritional factors (e.g. raffinose, stachyose). Classical and molecular breeding efforts, as well as meal processing procedures, mitigate these problems (Gu et al. 2010). Soybeans are used for human food, but it is the oil that serves as an important food ingredient. Soybeans contain healthy unsaturated omega 3, 6 and 9 fatty acids, but the oil profile has not been bred for broader uses until recently (United Soybean Board). Soybean oil has many industrial uses, including biofuel, lubricants and plastics. Significant breeding and biotechnology efforts have been directed at improving soybean oil profiles for food and industrial uses (Clemente and Cahoon 2009).

The genus *Glycine* consists of two subgenera, *Glycine* and *Soja*. Cultivated soybeans, *Glycine max*, belong to the subgenera *Soja* and commercial cultivars have a relatively narrow genetic base (Figs. 12.2 and 12.3). There are advantages to utilizing plant introductions (PIs) and wild relatives as sources of many desirable traits. *Glycine soja* Sieb. & Zucc. is an annual wild soybean and, like the perennial *G. tomentella* Hayata, has been used by soybean breeders as a source of genetic diversity, especially for yield and yield-preservation traits including disease and insect resistance genes (Hartman et al. 2000). The technical details of soybean breeding are described elsewhere (Lewers et al. 1996) and not intended to be the focus of this chapter.

Fig. 12.2 Typical phenotype of US commercial soybean cultivar. (Photo courtesy of Joseph Murphy, Iowa Soybean Association)

Fig. 12.3 Commercial soybean production showing minor insect feeding damage on leaves. (Photo courtesy of Joseph Murphy, Iowa Soybean Association)

Soybean breeding priorities for the past approximately 40 years have focused on yield, defensive traits, seed composition and quality. Soybean yields in the US since 1965 have increased from 1648 kg/ha (24.5 bu/ac) to 3228 kg/ha (48 bu/ac) in 2015 (USDA-NASS 2018) (Fig. 12.6). This yield increase is attributed to improved genetics and significant advances in agronomics and cropping systems, including precision and digital agriculture (Specht et al. 2014). Protecting soybeans from disease pathogens, insect pests and abiotic stressors has been especially important, as a number of fungal, bacterial and virus pathogens, nematodes, insect pests and abiotic problems like iron deficiency chlorosis impact US soybean production (Hartman et al. 2015b). Soybean researchers and breeders have successfully identified and introgressed native traits for resistance against many disease-causing pathogens. Several molecular genetics techniques, including the development of molecular markers and marker-assisted selection (MAS), streamlined and accelerated the development of disease- and insect pest-resistant soybean cultivars (Sebastian et al. 2012). In addition, improved agronomic practices and fungicides have enhanced disease management and soybean production. Insect pest control has largely been accomplished with insecticides, although some insects, including soybean aphids (*Aphis glycines*), are effectively managed with a combination of native resistance genes (*Rag*) and insecticides (Hesler et al. 2013).

The introduction of biotechnology-derived weed management in soybeans during the 1990s revolutionized weed control, agricultural biotechnology, reduced tillage and other agriculture conservation and management practices. The first commercial herbicide tolerant soybeans (Roundup Ready®) were engendered with a glyphosate (N-(phosphonomethyl) glycine) herbicide-resistant bacterial gene encoding the enzyme EPSP synthase (5-enolpyruvalshikimate-3-phosphate syn-

thase) (Padgette et al. 1995). Transgenic soybeans expressing the EPSP synthase gene are tolerant to over the top applications of the broad-spectrum post-emergent glyphosate herbicide. Since the mid-2000s, greater than 90% of all soybeans grown in the US are transgenic for herbicide tolerance (USDA-ERS 2017).

The modernization of weed management strategies for soybean and other crop production did more to change agriculture than any technology, process or practice since the Green Revolution. The development of transgenic herbicide-resistant soybeans led to increased soybean production, profitability and improved environmental sustainability by facilitating the use of environmentally-friendly herbicides, reduced tillage, lowered greenhouse gas emissions, and improved soil health, soil conservation and water quality. Furthermore, this advancement set the stage for other biotech crops including insect-resistant corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.).

Following the commercialization of biotechnology-developed herbicide-tolerant soybeans, new cultivars have recently been released with high oleic and low linolenic oil profiles (Demorest et al. 2016). Cultivars developed using ribonucleic acid interference (RNAi), antisense RNA (asRNA), transcription activator-like effector nuclease (TALEN) or mutagenesis and natural mutation selection technologies (Bilyeu et al. 2011; Demorest et al. 2016; USDA-APHIS 2011; Zhang et al. 2014), have the potential to expand the current industrial uses for soybean oil beyond biofuels, and improve the health and value of soybean oil in human food. The advent of new genome editing technologies like clustered regularly interspaced short palindromic repeats (CRISPR) will continue to revolutionize our ability to quickly, efficiently and precisely modify soybean traits for more productive soybeans with broader utility and greater health and nutritional value (Jacobs et al. 2015a).

Advances in molecular breeding and biotechnology, and the adoption of transgenic crops, changed the soybean industry in some unpredicted ways by impacting soybean breeding strategies and soybean cultivar development and commercialization. While private companies had begun to invest in soybean breeding programs in the 1970/1980s, coincident with the development of intellectual property protection laws, the high costs of developing and gaining regulatory approval for biotechnology traits accelerated private soybean commercialization and caused many universities and public institutions to shift their focus from cultivar development (e.g. through foundation and certified seed programs) to more basic research and discovery. This led to an increased focus on germplasm discovery, characterization and introgression of native traits for disease and insect resistance, increased emphasis on studies to elucidate the molecular mechanisms of plant-pathogen and plant-insect interactions, the development of robust breeding lines for the private sector, discovery of new technologies and tools, cooperative integration of multiple disciplines for soybean research, and the development and validation of agronomic practices and cropping systems that improve soybean production and profitability. Furthermore, many smaller soybean seed companies now license high value biotechnology traits from larger companies for direct commercialization and introgression into their elite breeding lines and commercial cultivars.

Today, in part due to the emergence of herbicide resistant weeds that jeopardize some of the transgenic herbicide tolerant soybean systems (Fig. 12.4), there is renewed interest in partnerships between the public and private sectors to discover, develop, evaluate and commercialize new technologies, tools and genetics for continuously improving the quality, productivity, profitability and sustainability of soybeans.

Ongoing research investments and partnerships among public, private and commodity organizations will ensure that soybeans continue to be a premier source of protein and oil for providing feed, fuel, fiber and food in safe and environmentally-sustainable ways for a growing world population. Remembering that soybeans and soybean production are often limited by biotic and abiotic stressors (i.e. pathogens, nematodes, insects, weeds, soil fertility, temperature, moisture), many opportunities remain for applying new and multi-disciplinary approaches for soybean improvement, genomics, breeding, agronomics and biotechnology efforts that build our understanding of gene function in determining plant growth and development, yield, seed composition and quality, and interactions with biotic and abiotic stressors and the environment. Multi-disciplinary basic and applied soybean research and efficient and effective public and private partnerships will lead to greater productivity, profitability, sustainability, new markets and new uses for soybean.

This soybean breeding chapter is organized into several sections written by recognized soybean research experts (see Contributor list). Sections include Breeding

Fig. 12.4 Appearance of waterhemp, *Amaranthus tubercalatus* (Moq.) J.D. Sauer, and other herbicide-resistant weeds in commercial soybean production field. (Photo courtesy of Joseph Murphy, Iowa Soybean Association)

for Yield, Agronomics and Cropping Systems, Yield Preservation from Pathogens, Insects and Abiotic Stressors, Germplasm Diversity and Conservation, Molecular Breeding, Mutation Breeding, Genetics, Genomics and Phenomics, Food Grade Breeding, Composition and Quality, and Applications for Bioinformatics, Computational Biology and other Analytics and Engineering Technologies. I (EJA) express my sincere appreciation to each of these authors for their time and expertise in meaningfully contributing to this chapter.

12.2 Soybean History, Introduction, Cultivation and Breeding for Enhanced Yield

12.2.1 Domestication and History

Soybean [*Glycine max* (L.) Merr.] was domesticated from *Glycine soja* Sieb. & Zucc., an annual species that grows wild in China, Japan, Korea, Russia and Taiwan (Hymowitz 2004). *Glycine soja* has a vining growth habit and produces small, black seeds in pods that shatter when mature. *Glycine soja* and soybean are mostly interfertile and artificial crosses can be made between the two species.

Where and when soybean was domesticated has not been proven, but evidence suggests that soybean was domesticated in China approximately 3100 years ago (Ho 1975). After domestication, soybean was cultivated in East Asian countries and was critical to human nutrition. The most important foods made from soybean in Asia include miso, soy sauce, tempeh and tofu. Until the twentieth century, soybean was largely an Asian crop but during the last half of the twentieth century and into the twenty-first century, soybean production increased dramatically in the Western Hemisphere, led by production in the US, Brazil, and Argentina (Wilcox 2004).

The first documented case of soybean grown in what is now the US was by Samuel Bowen, who grew the crop on his property outside of Savannah, Georgia in 1765 (Hymowitz and Harlan 1983). The production of soybean in the US remained insignificant until the twentieth century. Soybean was initially grown in the US as a forage crop and the area devoted to it for that purpose exceeded other uses until 1941. Area under soybean production doubled from 1941 to 1942, remained relatively constant through the 1940s and then grew dramatically from the 1950s until it peaked in 1979 (Fig. 12.5). The US production area expanded in the late 1990s and increased again recently. During the past 40 years, soybean has lead all major crops for annual increases in global production area (Hartman et al. 2011). In 2017, soybean was grown on 36.5 million ha (90 million ac) with a total production of 119.5 million mt (4.4 B bushels).

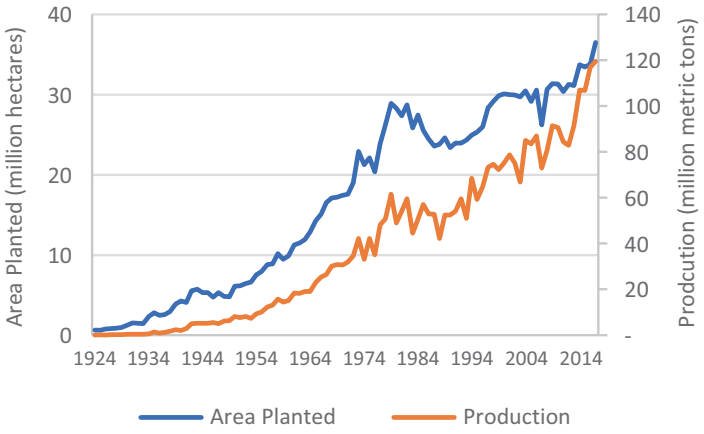

Fig. 12.5 Annual area planted to soybean and soybean grain production in the US 1924–2017. (Data were obtained from the National Agricultural Statistics Service website (USDA-NASS))

12.2.2 Soybeans in the United States

The US soybean production was initiated through introducing and growing cultivars from other countries. In 1939, Morse and Cartter described 108 soybean cultivars in the US and all were introductions from Asia, selections from introductions, or natural crosses in introductions. Upon the establishment of breeding programs, these introductions were used as parents that became the genetic basis of soybean cultivars in the US. An analysis of pedigrees show that 37 early introductions contributed 95% of the genes in the current genetic base of soybean cultivars in the US (Gizlice et al. 1994). Although early breeding efforts were small because of a lack of resources, including mechanization, an early cultivar release in the northern US was Lincoln, which had a 17% yield advantage over the cultivars it replaced (Hartwig 1973). This cultivar was widely used as a parent in breeding programs at that time and is estimated to provide 24% of the genetic base of cultivars in the northern US (Gizlice et al. 1994). The cultivar Lee had an even greater impact on soybean improvement in the southern US. Lee was released in 1953 and is estimated to provide 46% of the genetic base of cultivars in the southern US (Gizlice et al. 1994).

Farmers grew soybean cultivars bred largely in the public sector until the late 1970s to early 1980s when cultivars developed by the private sector became the predominant source of seed (Specht et al. 2014). A major factor driving this transition was the passing in 1970 of the Plant Variety Protection (PVP) Act, which spurred private sector investment in the seed industry (Fehr 1991). Even stronger incentives for private sector breeding occurred when utility patents were allowed for seed-propagated plants resulting in the patenting of the first soybean cultivar in 1994 (Mikel et al. 2010).

During the period 1924–2017, US average on-farm soybean yields increased from 739 to 3300 kg/ha (11 to 49 bu/ac) (USDA-NASS 2017) (Fig. 12.6). Using a linear model, the annual rate of gain calculates to 24.4 k/ha/year (0.36 bu/ac/year) for the entire period. For the 40-year period 1978–2017, the rate of gain increased to 33.4 kg/ha/year (0.5 bu/ac/yr). These yield gains are the result of improvements in genetics, agronomic practices, the production environment, and interactions between all three factors. A way to estimate the genetic contribution to yield gains is to grow soybean cultivars released in different eras in field tests in common environments. These genetic gain estimates have been made several times over the past few decades and the most recent estimate was published by Rincker et al. (2014). They conducted field tests during 2010–2011 with sets of 49–60 cultivars from maturity groups (MGs) II, III and IV that where released from 1923 to 2008. When a linear model was used, the researchers observed yield gains that ranged from 20–23 kg ha/year (0.3–0.35 bu/ac/year) for individual maturity group sets. A two-segmented model was found to be significantly more probable than a simple linear model for each maturity group. The breakpoints for the two segments ranged from 1964–1971 and the post breakpoint gains ranged from 26–31 kg ha/year (0.39–0.46 bu/ac/year). Although these estimates are only slightly below that of the on-farm yield improvements cited above, a thorough analysis of the data by Specht et al. (2014) estimated that approximately two-thirds of the US yield gains were the result of genetic improvements while the remainder from better agronomics and other causes. The increased rate of yield gain during the recent decades, as observed both on farm and in the genetic gain study, was likely the result of greater investments in plant breeding in the private sector after the passing of the PVP law.

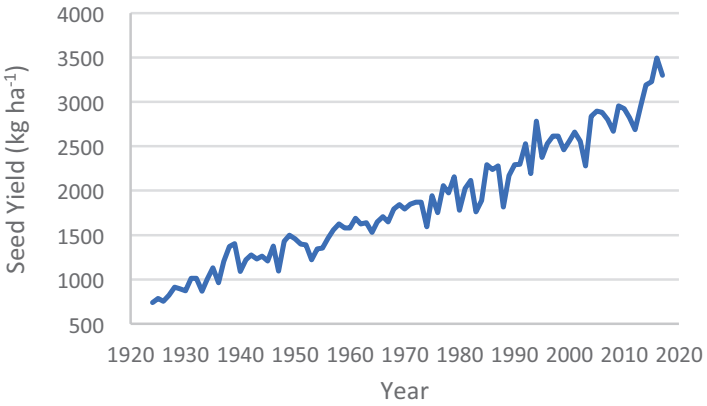

Fig. 12.6 Average on-farm soybean grain yields in the USA 1924–2017. Average yields were obtained from the National Agricultural Statistics Service website (https://www.nass.usda.gov/)

12.2.3 Breeding for Yield

Improving grain yield has been the major focus of breeding programs in the US because yield is the major factor soybean farmers consider when they select cultivars to grow. This is the result of soybean farmers being compensated largely based on the weight of grain they deliver to buyers. Although there is a major focus on yield, Rincker et al. (2014) observed significant trends for other traits across all three maturity group tests. When compared to old cultivars, they found that new cultivars matured later, had reduced lodging, reduced plant height, lower seed protein concentration and greater seed oil concentration. In addition, Hartman et al. (2015a) showed that there was a general improvement in disease resistance over generations of cultivar development. Many of these changes are likely the result of selection for greater yield, for example, seed protein has been shown in many studies to be negatively associated with yield (Wilson 2004)

The genetic improvement of soybean yield has largely been the result of conventional breeding efforts. Yield increases have been the result of repeated cycles of selection, which has resulted in a continued pyramiding of yield improving genes in new cultivars. The increased rate of gain observed during the last decades is likely largely due to the increased scale of the conventional breeding efforts over the past 40 years, especially in the private sector. Sophisticated plot planters and combines have increased the ability of breeders to evaluate more experimental lines, and improvements in data analysis techniques have made these data more valuable. Eathington et al. (2007) stated that Monsanto's investments in breeding programs had resulted in an 80% increase in plot capacity during the 4–5 years prior to the publication. It should be noted that the acceleration in yield gain observed has not matched the much greater increase in the scale of breeding programs, meaning that it is becoming more expensive to achieve genetic gains for yield.

The gains through conventional breeding have been aided by MAS and genomic selection. In soybean, MAS has been especially useful in the selection of disease and pest resistance because of the relatively simple inheritance of these traits and the availability of markers tightly linked to resistance genes (Cahill and Schmidt 2004). In contrast, it has been much more difficult to successfully implement MAS to improve grain yield because of the complexity of inheritance of this trait and the impact of background genes and the environment on genes controlling yield. Genomic selection holds the promise of increasing gains by not selecting for individual markers, as with MAS, but with whole genome prediction through the simultaneous use of information from markers throughout the genome (Jarquin et al. 2014). It is expected that continued innovations in breeding technology and genetic technology should help propel future gains.

12.3 Production, Agronomics and Cropping Systems

12.3.1 The US North Central Region

The US accounts for almost 34% of the global soybean production, producing 106 M mt (3.9 B bu) annually (FAOSTAT & USDA-NASS 2013–2016). Of that total, 82% is produced in the North-Central region, where a 2-yr corn-soybean rotation is the dominant cropping system and includes 26 M ha (64.2 Mac) planted with soybean (Fig. 12.7a). Annual rainfall decreases almost linearly from east to west across the North Central, with a parallel increase in the evaporative demand. Hence, the probability and severity of water stress during the soybean growing season increases along the east-west transect. Soils are generally deep, fertile, rich in organic carbon, and have large water-holding capacity. By soybean planting time, the soil profile is typically filled to field capacity by cumulative rainfall in the fallow period between fall harvest and spring planting. In wet years, transient early-season waterlogging is likely in soils with moderate-to-low infiltration rates in the central and eastern regions of the Corn Belt, where subsurface tile drainage is currently used on about one third of total cropland to mitigate this problem (Sugg 2007). The western edge of the region includes the eastern Great Plains states of North Dakota, South Dakota, Nebraska and Kansas. Irrigation is fundamental in this region to ensure high yields with low interannual variation because growing-season rainfall and stored available soil water at planting are typically not sufficient to satisfy total crop water requirements (Grassini et al. 2014b, 2015). For example, irrigated agriculture accounts for 54% and 48% of the total soybean production and total area in Nebraska, respectively (Fig. 12.7b). A detailed description about corn-soybean systems in the US North Central region can be found in Grassini et al. (2014a).

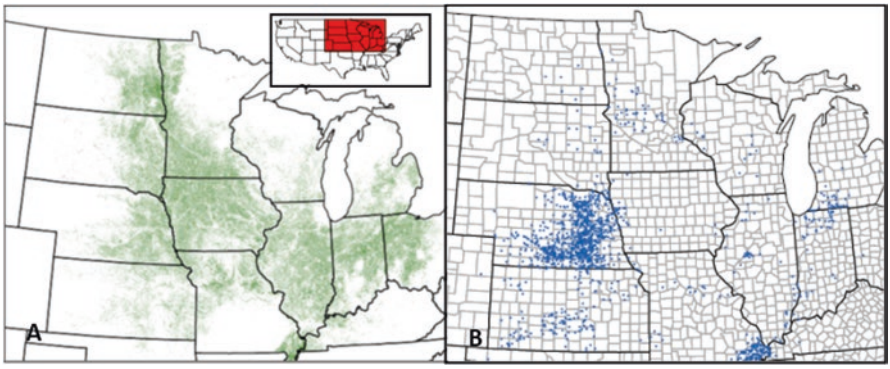

Fig. 12.7 Map of the US North Central region showing harvested soybean area (**a**) and irrigated area (**b**); one dot represents 810 ha (2000 ac). Total harvested area (2013–2016 average) was 26 Mha (64.2Mac) for soybean. State boundaries are shown for Iowa (IA), Illinois (IL), Indiana (IN), Kansas (KS), Michigan (MI), Minnesota (MN), Missouri (MO), Nebraska (NE) North Dakota (ND), Ohio (OH), South Dakota (SD), and Wisconsin (WI)). (Source: USDA-NASS)

12.3.2 Soybean Management

Soybean management is adjusted according to thermal and water regimes. The length of crop growing season is technically defined (on a calendar date basis) by the probability of a late spring frost occurring on or after crop emergence date and by the probability of an early frost before physiological maturity date. Average planting date for soybean ranges from early May in southern locations to late May in northern locations (Fig. 12.8a). Recommended North Central soybean maturity group (MG, range from 00 to IV) decreases from south-north, concordant with the long-term probability of frost occurrence during the final phases of seed filling (Fig. 12.8b). Planting date delays in soybean are associated with a linear yield

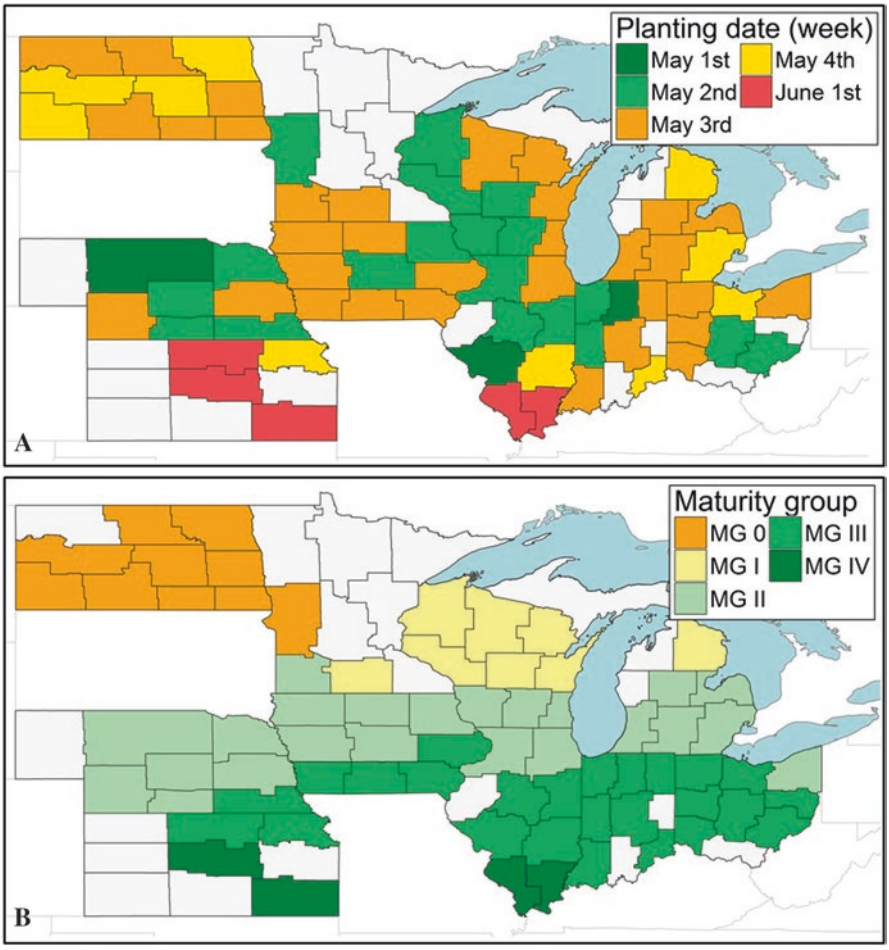

Fig. 12.8 Average planting date (**a**) and maturity group (**b**) per agricultural district across 10 states in the US North Central region. (Source: Mourtzinis et al. 2018; Rattalino Edreira et al. 2017)

penalty ranging from 0 up to 42 kg/ha/d (0.63 bu/ac/d) after May 1 depending on the degree of water limitation of the production environment (Bastidas et al. 2008; Rattalino Edreira et al. 2017; Villamil et al. 2012). A detailed description of soybean practices and inputs can be found in Grassini et al. (2015) and Mourtzinis et al. (2018).

On-farm average soybean plant density is typically 35–45 plants/m^2 (140,000–180,000 plants/ac), which is higher than required plant density for maximum yields (25–35 plants/m^2 or 100,000–140,000 plants/ac) due to lower soybean seed cost compared with hybrid corn seed, and varies little across regions or water regimes (De Bruin and Pedersen 2009; Mourtzinis et al. 2018). In many parts of the North Central region, there has been a shift during past decades from continuous corn under conventional tillage to a 2-yr corn-soybean rotation under reduced tillage (defined as any tillage method that leaves ≥ 30% of soil surface covered by plant residues). More than half of the total area planted with soybean in the North Central region is under reduced till practices (Mourtzinis et al. 2018). Soil residue cover with reduced till minimizes soil erosion, increases precipitation-storage efficiency during the non-growing season, and reduces surface runoff and soil evaporation. A small (<20%) number of soybean fields receive nitrogen (N), phosphorous (P) and potassium (K) fertilizer inputs. Soybean largely relies on mineralization of soil organic matter (~50%) and symbiotic N fixation (~50%) to meet N requirements and residual P and K fertilizer from the previous corn crop (Salvagiotti et al. 2009). Herbicide use is higher than fungicide and insecticide inputs due to cold winters, which interrupt the cycle of most insect pests and disease pathogens, and the widespread adoption of transgenic herbicide-resistance cultivars. In fact, 94% of the soybean fields in this region are planted with genotypes possessing herbicide-resistance traits (USDA-ERS 2014–2017). Most fungicides and insecticides are applied as seed treatment (>90% soybean fields) but foliar applications are becoming increasingly popular (20–40% soybean fields), even in the absence of pathogens or insect pests (Mourtzinis et al. 2018; Villamil et al. 2012).

12.3.3 Time Trends in Yields and Input-Use Efficiency

As previously described, rates of increase in average soybean yield in the North Central region have been markedly linear between 1964 and 2017. Annual rate of yield gain (i.e., slope of regression) decreased and interannual yield variation (r^2) increases from favorable to less favorable environments for crop production (e.g. Nebraska irrigated > Iowa, Illinois and Minnesota rainfed > Nebraska rainfed). Yield trends also show that the average difference between rainfed and irrigated soybean in Nebraska during the 1964–2017 interval was, on average, 0.8 Mg/ha (12 bu/ac) and, even in the best years for rainfed crops, this yield difference was never smaller than 0.3 Mg/ha (4.5 bu/ac) (Fig. 12.9, left panels). In the western part of the North Central region, allocation of the best land to irrigated agriculture, lower applied inputs and suboptimal water supply distribution during the growing season

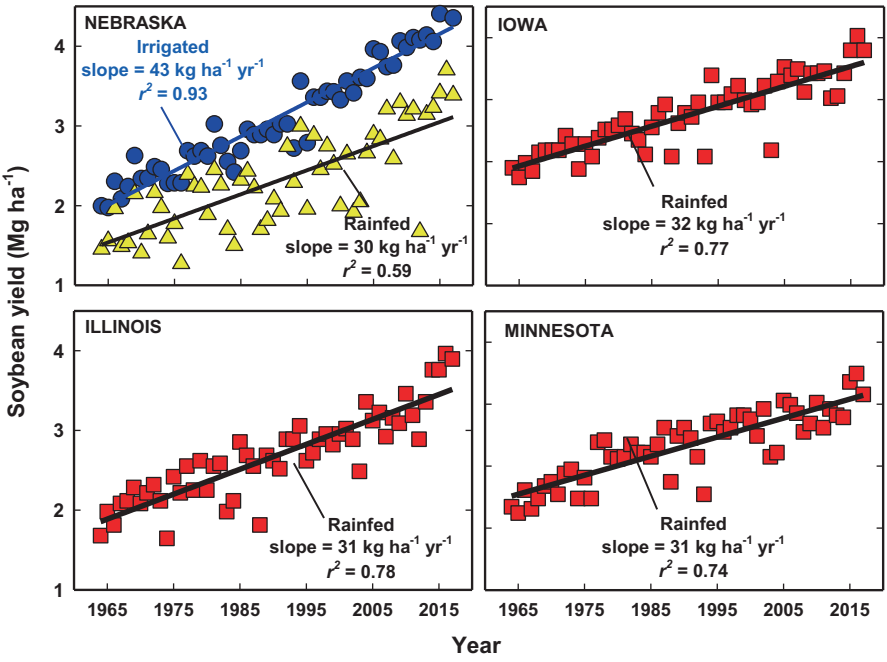

Fig. 12.9 Trends in soybean yield for four major US producing states (Nebraska, Iowa, Illinois, and Minnesota). These four states account for 43% of total US soybean production. Separate trends are shown for rainfed (triangles) and irrigated crops (circles) in Nebraska. (Source: USDA-NASS)

impose a limit to rainfed crop yields, even in years when growing-season rainfall can support yield above the long-term trend.

Improvement in crop yield arises from the adoption by producers of genetic technology in the form of newly-released soybean cultivars that have greater genetic yield potential and agronomic technology in the form of crop management practices that enhance the yield of the production environment. Synergistic genetic x agronomic interactions are also important, given that the greater genetic yield potential available in modern vs. obsolete cultivars is coupled with the greater yield of modern vs. dated agronomic practices (e.g. Rowntree et al. 2013). Agronomic drivers for yield gains; genetic drivers, and their interactions with agronomic practices, are reviewed by Grassini et al. (2014b) and Specht et al. (2014).

There are several agronomic practices that have contributed to higher soybean yields. In addition to irrigation and reduced tillage, soybean farmers have halved their row spacing from 0.76–0.38 m (30–15 in). Some farmers tried, but most moved away for narrower 0.19 m (7 in) rows, probably because drill-planters do not work well in heavy residue, no-till systems. Soybean farmers have persistently, over the past three decades, shifted their planting times to earlier calendar dates at a rate of about 0.4 d/yr. This shift may be attributable to climate change (i.e., warmer early

springs), genetics (better early-season germination and seedling cold tolerance in new cultivars), and agronomic technologies such as multi-row planters that lessened the number of total planting days and improved seed treatments that mitigate pathogen losses (Gaspar et al. 2017; Mourtzinis et al. 2015; Sacks and Kucharik 2011; Specht et al. 2014). Planting soybeans early to take advantage of warmer springs is one such practice that has contributed to the yield gains shown in Fig. 12.9. Soybean yield can theoretically be enhanced by 17–42 kg/ha (0.25–0.65 bu/ac) for every day that planting can be moved closer to May 1 for MG II & III cultivars grown in the North Central region [see Bastidas et al. (2008) for Nebraska data and references therein for other north central states]. Using a soybean simulation model (SoySim.unl.edu, Setiyono et al. 2010), Specht et al. (2014) estimated the yield impact if Nebraska soybean farmers had not advanced the irrigated soybean planting date. These authors documented that the planting date advance of 0.4 d/yr may have accounted for 1/8–1/5 of the observed on-farm irrigated yield gain of 54 k/ha/yr (0.8 bu/ac/yr) for irrigated soybean in Nebraska that concurrently occurred in the same 1983–2012 timeframe. More agronomic yield enhancement could be achieved if the 50% planting progress date were advanced more uniformly to May 1 or late April.

A soybean crop following corn in a 2-yr corn-soybean rotation out-yields soybean in monoculture (Fox et al. 2014 and references cited therein). Almost all soybean hectares (acres) are rotated with corn in the North Central region. It is noteworthy, however, that when a soybean crop follows two or more successive corn crops, the yield of the soybean crop is even greater than when soybean follows one prior year of corn (Farmaha et al. 2016; Fox et al. 2014). Crookston et al. (1991) found that, relative to monoculture soybean, annually rotated soybeans (with corn) yielded 8% more, and soybeans after 5 years of corn yielded 17% more. There is also a yield advantage of corn grown in rotation with soybean compared with continuous corn, in both irrigated and rainfed production systems. This yield advantage ranges from 2–15% in both experimental plots and producer fields (Farmaha et al. 2016 and references cited therein). Trends toward a greater proportion of total corn area in soybean-corn rotation, rather than continuous corn, has been estimated to account for 8% of corn yield gain in U.S. Corn Belt since 1970 (Farmaha et al. 2016).

Almost all soybeans grown in the North Central region are transgenic cultivars, and the vast majority of those cultivars possess a transgene that provides tolerance to the glyphosate herbicide. The use of glyphosate-tolerant cultivars has not only provided near-total weed control (though glyphosate-resistant weeds are becoming a significant problem), but it has also accelerated producer adoption of reduced- or no-till practices as well as the narrowing of row spacing. Owing to widespread use of glyphosate in herbicide-tolerant corn and soybean, weed resistance is becoming a substantial problem (Mortensen et al. 2012).

12.3.4 Current Yield Gap Due to Management

Soybean yield potential can be taken as a benchmark to estimate yield gaps, that is, the difference between average on-farm yield and the yield potential defined by solar radiation, temperature and genotype, as well as water availability in rainfed crop systems (Lobell et al. 2009; Van Ittersum et al. 2013). This definition of yield potential reflects an upper biophysical limit to what might be ultimately attainable for soybean yields on a given field; hence, the magnitude of the yield gap estimates the degree of yield improvement that could still be captured on that farm with adjustments in crop management. Specht et al. (1999) and Sinclair and Rufty (2012) reported that a yield potential of approximately 6 Mg/ha (90 bu/ac) for soybean can be used as a *functional* upper yield limit for on-farm average soybean yields. Using this benchmark and based on an average (2001–2010) irrigated soybean yield of 3.8 Mg/ha (57 bu/ac), the yield gap of irrigated soybean in Nebraska is about 37% of the potential. However, on-farm soybean yields of approximately 6 Mg/ha (90 bu/ac) might be achieved but only under the best possible genotype × location × year × management interaction across a large geographic area (e.g. Cafaro La Menza et al. 2017). Based on crop modeling and analysis of producer yield data, Grassini et al. (2015) found the average yield gap in Nebraska to range from 10–30% of estimated yield potential, with the latter ranging from 5.1–5.9 Mg/ha (76–89 bu/ac). Rattalino Edreira et al. (2017) extended this analysis to the entire North Central region, estimating an average yield potential of 4.8 Mg/ha (72 bu/ac) (rainfed) and 5.7 Mg/ha (85 bu/ac) (irrigated), with a respective yield gap of 22 and 13% of yield potential. These findings indicate a relatively small (but still exploitable) opportunity for increasing farmer soybean yields through fine-tuning of current management practices. A comprehensive analysis of yield gaps and their causes can be found in Rattalino Edreira et al. (2017) and Mourtzinis et al. (2018).

12.4 Abiotic and Biotic Stress Tolerance

Soybeans are subjected to various adverse abiotic and biotic stressors which substantially reduce soybean yield and quality. The major abiotic stressors include drought, flood and salinity, while the biotic stressors include weeds, various diseases caused by fungi, bacteria, viruses and nematodes, and several insect pests. Greenhouse and field studies show that drought stress causes significant reduction in seed yield (24–50%) (Sadeghipour and Abbasi 2012). Flooding causes significant losses, up to an estimated annual average of USD 1.5 billion (Bailey-Serres et al. 2012; GISS/NASS 2002). Saline and sodic soils were estimated as 397 and 434 million ha worldwide, respectively (FAO/AGL 2000 www.giss.nasa.gov/research/news/20021028/). It has been reported that 19.5% of irrigated land and 2.1% of dryland production are affected by salt stress. These salt-affected lands cover about 20% of the world's food production and consumption (Pimentel et al. 2004).

Similarly, disease and insect pressures cause significant annual soybean losses. In North America, annual losses to soybean diseases are estimated to be greater than 8.8 M MT (400 M bu) (Bradley et. al. 2015). Depending on location and year, soybean yield loss due to insect pests can exceed 40% (USDA-NAAS 2014).

Stress-, pathogen- and pest-tolerant plants evolve through natural selection by acquiring acclimation and through adaptation mechanisms. Exotic and wild relatives that grow in a wide range of geographical areas and climatic conditions often possess better stress-tolerance characteristics than native cultivars (Mickelbart et al. 2015). Introduction of these genetic resources into crop germplasm increases diversity and supports the sustainability of crop production. With a view to offset the soybean crop losses due to abiotic and biotic stressors, soybean breeders have been making continued progress to develop new soybean cultivars with tolerance to different stressors using conventional as well as molecular breeding approaches.

12.4.1 Abiotic Stressors

12.4.1.1 Drought

Drought during vegetative stages causes soybean leaves to wilt, curl or drop, leading to reduced plant growth and yield. Drought during reproductive stages results in flower and pod abortion, and smaller pods with fewer, smaller and shriveled seeds (Boyer 1983). Drought tolerance is a complex trait involving many morpho-physiological traits, including root characters (Ludlow and Muchow 1990). It can be achieved via drought avoidance or desiccation prevention, a combination of these, through effective use of a limited water supply, or recovery of growth with rehydration after drought stress (Chaves et al. 2003; Passioura 2012). A deep, thick root system with extensive branching is considered a major component of drought avoidance, enabling plants to extract water from deep soil layers (Fukai and Cooper 1995; Gowda et al. 2011). Other beneficial traits include slow canopy wilting and sustained N fixation under drought. A simulation analysis model predicted that slow canopy wilting and sustained N fixation can improve yield under drought by >75% and 85%, respectively (Sinclair et al. 2010).

Traditional breeding approaches utilize natural genetic variation available in the existing germplasm. Crosses are often made between drought tolerant germplasm with desired traits and adapted high-yielding cultivars. Progenies that exhibit drought tolerance under drought conditions are selected and evaluated for yield under drought and irrigated conditions. Drought indexes are calculated by dividing drought yield by irrigated yield and then multiplication by 100 (Pathan et al. 2014). Based on the yield performance with and without irrigation (high index values), the best breeding lines are chosen and used to develop new cultivars. In many US soybean breeding programs, drought tolerant lines are selected based on the canopy wilting score (1–5 scale, where 1 = no wilting to slow-wilting, and 5 = severe wilting, fast wilting, or plant death) (Pathan et al. 2014). As an example, the University

Fig. 12.10 Side-by-side comparisons of drought-sensitive and drought-tolerant, slow-wilting soybean. (Photo courtesy of P. Chen)

of Missouri soybean breeding program developed several drought tolerant breeding lines using slow wilting exotic landraces (PI 567690, PI 567731) (Pathan et al. 2014). Following a traditional approach, the University of Arkansas soybean breeding program released several germplasm lines that have drought tolerance and prolonged N-fixation capacity under drought, such as R01-416F, R01-581F (Chen et al. 2007) and R02-1325, R05-5559, R07-5235 (Devi et al. 2014). Recently, the USDA-ARS released a conventional maturity group VIII soybean cv. N8002 which has drought tolerance and high-yield potential under stress conditions (Carter et al. 2016).

With conventional breeding methods, selection of drought-tolerant breeding lines is done under artificial greenhouse or growth chamber conditions, or natural field conditions, where the occurrence, timing and severity of drought is uncertain (Fig. 12.10). MAS can accelerate the development of drought-tolerant cultivars. Drought tolerance is a quantitative trait, controlled by many genes. Through molecular mapping, genes or quantitative trait loci (QTL) controlling drought tolerance are identified and mapped, and the QTL-linked DNA markers are used in MAS. Recently, QTLs for drought tolerance related traits, such as slow wilting (Abdel-Haleem et al. 2012; Hwang et al. 2016); root length (Prince et al 2015) and steeper root angle (Fenta et al. 2014) in soybean have been reported. With the development of next generation genomic technologies, plant breeders have recently started using high-resolution genomic information for germplasm characterization, genetic diversity analysis, genetic dissection of major agronomic traits, and development of user-friendly QTL-linked markers for more efficient selection of improved breeding lines.

With the advancement of biotechnology and availability of genome sequence information, germplasm resources and newer genomic tools, the transgenic approach is becoming an attractive alternative strategy in breeding for improved cultivars. This approach relies on the identification of desired candidate genes that can improve drought tolerance but do not cause yield reduction when incorporated into

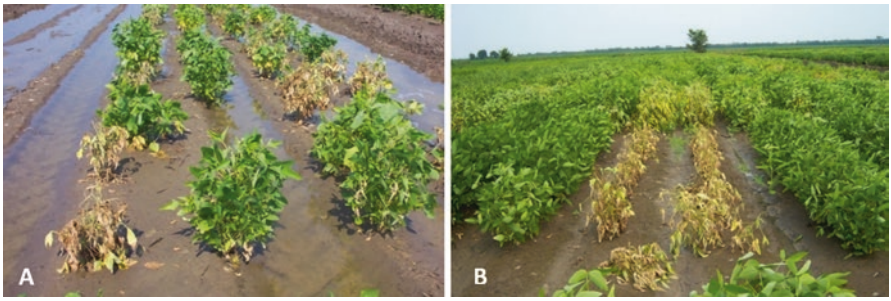

Fig. 12.11 Field screening and selection for flood tolerance in soybean. **a** Hill plots of germplasm lines and genetic populations flooded for 5-7 days, followed by flood injury and plant death scoring and observations, **b** Two-row plots of breeding lines as part of a large flooding experiment for flood tolerance selection. (Photos courtesy of P. Chen)

drought-sensitive cultivars. Transgenic soybean expressing the *AtP5CR* gene (encoding L-Δ1-Pyrroline-5-carboxylate reductase) resulted in enhanced drought tolerance under test conditions (de Ronde et al. 2004).

12.4.1.2 Flooding

Flooding ranks second after drought among the abiotic stressors causing the highest economic losses in soybean production (Mittler 2006). Flooding or waterlogging causes reduction in root growth, shoot growth, nodulation, nitrogen fixation, photosynthesis, biomass accumulation, stomatal conductance, nutrient uptake and disease resistance (Oosterhuis et al. 1990). Waterlogging during early vegetative or reproductive growth, for periods as short as 2 days in clay soils, can reduce yield by as much as 27% (Linkemer et al. 1998; Tamang et al. 2014). Therefore, it is important to develop flood tolerant soybean cultivars for high yield in regions with heavy rainfall and poorly drained soils (Fig. 12.11). Flooding tolerance is also a complex trait involving various physiological factors including sustained root and shoot growth, water uptake and respiration in anaerobic environments, dehydration tolerance that supports plant survival under waterlogging or water submergence and enable quick recovery from flood injury after flood waters recede.

Exotic germplasm and wild soybeans are excellent sources for flooding tolerance. Shannon et al. (2005) screened a core set of 350 soybean germplasm lines for flooding tolerance during early reproductive stages and found a number of tolerant lines, including Archer, Misuzudaiz, PI 408105A, PI 561271, PI 567651 and PI567343. They also found some wild soybeans (*Glycine soja*) that showed excellent flooding tolerance. The University of Missouri soybean breeding program developed breeding lines from the crosses with flooding tolerant PI 408105A that also showed good yield potential. Wu et al. (2017) evaluated 722 soybean genotypes for flooding tolerance for 5 years (2012–2016) and identified 11 genotypes that consistently exhibited high levels of flooding tolerance. Following a conventional

breeding approach, the Arkansas Agricultural Experiment Station released several flood tolerant soybean cultivars, especially UA 5612 and UA 5615.

Nguyen and colleagues mapped two major flooding tolerance QTLs – on chromosome 11 (*FTS-11*) and 13 *(FTS-13*) using the mapping population S99-2281 × PI 408105A (Nguyen et al. 2012). The major QTL *FTS-13* from the flooding tolerant germplasm PI 408105A accounts for up to 18% of the phenotypic variation. DNA markers flanking this QTL have been developed for MAS. Two flooding tolerance QTLs have also been mapped, one on Chromosome 3 (*FTS-03*) and the other one on chromosome 10 (*FTS-10*) in the population S99-2281 × PI561271. *FTS-03* is a major QTL from PI 561271, accounting for up to 33.1% of the phenotypic variation. Several near isogenic lines (NILs) containing *FTS-3* QTL exhibited greater yield than the NILs lacking the flooding tolerance QTL under flooding stress condition.

12.4.1.3 Salinity

Soybean yield is reduced by soil salinity with a concentration exceeding 5 dS/m (Ashraf and Wu 1994) and yields can be reduced by 40% due to salt stress (Papiernik et al. 2005). Salt injury symptoms include leaf scorching and a reduction in plant size, number of internodes, number of branches, number of pods and seed weight (Chang et al. 1994). Salinity affects growth by imposing both osmotic and ionic stress. Soil water potential is decreased due to the presence of high concentrations of salt, thus making it hard for the roots to take up water from soil. The ionic stress is associated with a gradual accumulation of salts in plant tissues over time (Munns and Tester 2008). Salt tolerance in plants involves complex physiological traits, metabolic pathways and molecular mechanisms (Gupta and Huang 2014). Research revealed three major mechanisms for salt tolerance in plants including ion (chloride) exclusion in roots, osmotic tolerance and tissue tolerance (Roy et al. 2014). Chloride exclusion is primarily used in breeding for salt tolerance.

Substantial natural genetic variation exits for salinity tolerance in the soybean gene pool. Salt tolerance screening is done at V1 (vegetative growth stage 1) in a 100 mM salt solution and salt injury is scored when the susceptible check Hutcheson cv. shows chloride toxicity symptoms on the leaves. Salt tolerance scoring uses a scale of 1 to 5 where 1 = no injury and 5 = dead plants (Lee et al. 2004; Valencia et al. 2008). Sensitive soybeans are referred to as chloride includers, such as cvs. Williams, Clark, HBK R4924, and Dare while tolerant lines, referred as chloride excluders, such as S-100, Lee 68, and HBK R5525 (Valencia et al. 2008). During the last five years, universities have released several high yielding soybean cultivars with salt tolerance.

Salt tolerance screening under field and greenhouse conditions is time-consuming, unpredictable and unreliable. However, molecular markers have proved to be effective in selection for salt tolerance. Several major QTLs conferring salt tolerance have been identified. A major QTL near the simple sequence repeat (SSR) markers Sat_091 and Satt237 on chromosome 3 was detected in three different populations (Hamwieh et al. 2011; Lee et al. 2004). Chen et al. (2008) identified a major

QTL between SSR markers Sat_164 and Sat_358 on chromosome 18 based on phenotyping in the field and greenhouse. Using cv. Osage, Zeng et al. (2017) reported a major QTL on chromosome 3 and two minor QTLs on chromosomes 13 and 15. Shi et al. (2018) reported a major QTL from cv. Jidou 12, flanked by SSR markers GMABAB and Barcsoyssr_03_1421 on chromosome 3 with R^2 values of 44.3–44.7%.

Salt tolerance gene *Ncl* from a wild soybean was transferred to a salt-sensitive cv. Jackson through MAS (Do et al. 2016), and the introgressed line yielded higher than the sensitive check cultivars in saline field conditions. The *Ncl* gene was found to be expressed in transgenic soybean lines and exhibited tolerance to salinity (Do et al. 2016). Ren et al. (2016) reported that soybean salt tolerance gene 1, named *GmST1*, exhibited strong tolerance to salt stress in transgenic *Arabidopsis*, indicating its reliability for MAS or for developing transgenic soybeans.

12.4.2 Biotic Stressors

Soybeans are affected by a wide range of weeds, arthropod pests and disease pathogens which can severely limit yield (Hartman et al. 2015b; Higley and Boethel 1994). Pests include aphids, beetles, lepidopteran caterpillars, mites, stink bugs, and numerous pathogens including fungi, bacteria, viruses and nematodes. Conventional and marker-assisted breeding and genetic modification efforts have produced many soybean cultivars with resistance to insects (e.g., soybean aphid, soybean looper moth, Mexican bean beetle) and diseases (e.g., soybean cyst nematode, *Phytophthora* root and stem rot, *Sclerotinia* stem rot) (Chang and Hartman 2017; Chawla et al. 2013). Resistance may take the form of antixenosis (non-preference or repellency), antibiosis (causing insect mortality or reduced population growth) or tolerance (mitigated yield loss in the face of pest pressure) (Painter 1941; Parrott et al. 2008).

Breeding for pathogen and insect pest resistance in soybean involves several facets and opportunities for the convergence of multiple disciplines and expertise. First, it is important to note that most plants are not affected or negatively impacted by most microorganisms and insects. Where infections and infestations do develop, pathogenic microorganisms and insect pests cause disease and damage that can range from minor to devastating. The reaction following soybean infection by a pathogen depends upon the genetics of the host and the pathogen, as well as environmental conditions (Francl 2001). Working together, soybean breeders, pathologists, entomologists and molecular biologists have identified some interesting plant-pathogen and plant-insect interactions that have aided in the selection and screening of native, or naturally occurring, resistance genes. In addition, these collaborations have led to the conception of some novel biotechnology-based control and management strategies.

A comprehensive review of pest resistance in soybean is beyond the scope of this chapter. Instead, our intent is to emphasize through case studies (1) the important role

that resistance plays in integrated pest management (IPM) in soybean, and (2) that resistance traits are finite resources in the face of pest evolution to overcome this resistance.

12.4.2.1 Diseases

Resistance, or *R,* genes have been identified in soybean breeding lines, plant introductions, wild relatives and exotic germplasm for introgression and management of viruses (alfalfa mosaic, soybean mosaic, bean pod mottle), bacteria (*Pseudomonas syringae*, *Xantomonas axonopodis* pv. *glycines*), fungi (*Cercospora kikuchii*, *C. sojina*, *Peronospora manshurica*, *Microsphaera diffusa*, *Septoria glycines*, *Phakopsora pachyrhizi*, *Colletotrichum truncatum*, *Phialophora gregata*, *Macrophomina phaseolina*, *Fusarium* sp., *Phytophthora sojae*, *Pythium* sp., *Rhizoctonia solani, Sclerotinia sclerotiorum*), soybean cyst nematode (SCN; *Heterodera glycines*) and sudden death syndrome (SDS; *Fusarium virguliforme*) (Mueller et al. 2010). In several cases, resistance genes have been identified in the germplasm but not yet introgressed into commercial cultivars. These genes may provide tolerance, partial resistance or strong resistance, and may function as single, dominant genes (race-specific, monogenic, qualitative or vertical resistance), alleles of single genes or as multiple genes (partial, polygenic, quantitative or horizontal resistance) that work together to provide weaker but more durable resistance (Agrios 2005).

12.4.2.2 Nematodes

Three nematode groups, soybean cyst nematode (SCN, *Heterodera glycine*), root knot nematode (RKN, *Meloidogyne incognita*) and reniform nematode (RN, *Rotylenchulus reniformis*), are destructive soybean pathogens, in addition to bacterial, fugal, and viral diseases. Host plant resistance has played an important role in their control (Kim et al. 2016a). By screening exotic and native soybean germplasm, resistance sources have been identified and used in breeding programs in the US (Vuong et al. 2013). Nematode resistance is polygenic, and a number of genes/QTLs have been identified (Kim et al. 2016a). Concibido et al. (1997) mapped a major QTL, *Rhg1* for resistance to SCN HG types 2.5.7 (race 1) and 0 (race 3) on Chromosome 18. Recent studies revealed that the strength and durability of Rhg1 resistance is determined by its copy number (Lee et al. 2015c). The second major QTL, *Rhg4* associated with SCN HG type 0 (race 3) resistance has been mapped on Chromosome 8 in various germplasm sources including PI438489B (Vuong et al 2011). Another SCN-resistance QTL (qSCN11) on Chromosome 11 in several genetic backgrounds including PI 438489B has been reported (Abdel-Majid et al. 2014). Liu et al. (2012b) successfully cloned the second major QTL, *Rhg4*, from cv. Forrest. Three RKN-resistance QTLs on Chromosome 10 (Xu et al. 2013; Jiao et al. 2015) and on Chromosomes 17 and 13 (Jiao et al. 2015) have been mapped and reported. Lee et al. (2015a) identified 7 germplasm with resistance to RN from a

screening of 120 soybean accessions. Three RN-resistance QTLs have been mapped on Chromosome 11, 18 and 19 (Ha et al. 2007; Jiao et al. 2015). Recently, the University of Missouri soybean program released two high-yielding cultivars, namely S11-20124 and S14-9017 with multiple nematode resistance. However, when sources of resistance to any given pest are limited in number and widely deployed, the potential exists for pests to evolve virulence to overcome plant resistance, which is an ongoing concern with pathogenic nematodes.

12.4.2.3 Aphids

The soybean aphid is native to Asia and became a significant invasive pest in North America shortly after its first detection in 2000 (Ragsdale et al. 2011). Soybean aphids are phloem-feeding insects which use their piercing mouthparts to suck soybean sap, which can cause stunting, reduced or aborted pods, and (under heavy infestation) yield loss exceeding 40% (Tilmon et al. 2011). In addition, they may transmit soybean viruses (e.g. soybean mosaic virus) (Wang et al. 2006). Estimates of potential economic loss from this pest in the US in the absence of effective host plant resistance range from USD 3.6 –4.9 billion (Kim et al. 2008). In the face of widespread distribution and infestation in the U.S. in the early twenty-first century, insecticides became the first line of defense resulting in a 130-fold increase in insecticide use in soybean (Ragsdale et al. 2011).

The discovery of soybean aphid resistance genes and subsequent breeding efforts to incorporate these genes into agronomically-viable cultivars has been a valuable contribution to soybean IPM. Not only do aphid-resistant cultivars reduce the need for insecticide, but they also thereby provide a more hospitable environment for biological control agents such as ladybeetles and parasitoids which help limit aphid populations. Several sources of resistance to soybean aphid (*Rag* genes) were first found in 2004 (Hill et al. 2004a,b) and subsequently at least eight different genes have been mapped to four chromosomes (Hesler et al. 2013). The *Rag* genes confer antibiosis, significantly reducing soybean aphid survival and reproduction. For example, a pyramid of *Rag1+Rag2* has been found to maintain soybean aphid below the economic injury level without recourse to insecticide (Hesler et al. 2013; McCarville et al. 2014). Despite limited commercial adoption to date, the availability of these sources of aphid resistance is a very valuable potential component to environmentally and economically-sound pest management in soybean.

Yet, host plant resistance is a resource which can diminish as pests evolve the ability to overcome it. Virulent soybean aphid biotypes (capable of survival and reproduction on resistant cultivars) were first reported by Kim et al. (2008). Currently there are four known biotypes of soybean aphid, three of which are virulent against certain *Rag* cultivars including the *Rag1+Rag2* pyramid (Alt and Ryan-Mahmutagic 2013). Such biotypes have been found in a number of US locations at varying frequencies (O'Neal et al. 2018) and population genetic analysis suggests either an epigenetic basis or a widely distributed gene complex (Wenger and Michel 2013). Insect resistance management (IRM) strategies, such as the use of a

susceptible refuge, have the potential to slow pest evolution to host plant resistance thus increasing the longevity of resistant cultivars in pest management programs. Research is currently being conducted to develop IRM for soybean aphids and *Rag* genes with the goal of ensuring the long-term potential of resistant cultivars to contribute to sustainable pest management in soybean.

12.4.2.4 Weeds

Soybean row crop production under standard US cropping systems presents many opportunities for weedy species to compete for nutrients, water and solar radiation. Many species of broadleaf and grassy weeds combine to cause approximately 20% annual losses to soybean yield quantity and quality (Dille et al. 2016). Conventional weed control primarily includes tillage and the use of chemical herbicides. While effective for weed control, tillage can negatively impact soil structure and fertility, contribute to soil compaction and erosion, increase nutrient loss, negatively impact water management and quality, limit beneficial agronomic practices like the use of narrow row spacing, and increase greenhouse gas emissions. Chemical herbicides contribute significantly to weed management but are associated with collateral problems including crop plant toxicity, negative impacts on off-target plants, the development of herbicide-resistant weeds (Fig. 12.4), and toxicity to humans, animals, insects and soil microbes through direct exposure, unintended drift and introduction into water and food supplies.

Soybean breeding for weed management became feasible through agricultural biotechnology and the incorporation and expression of an altered glyphosate-tolerant EPSP (5-enolpyruvylshikimate-3-phosphate) synthase gene in transgenic soybeans (Padgette et al. 1995). Since the first commercial glyphosate (N-(phosphonomethyl) glycine) tolerant soybeans in 1996, soybeans tolerant to glufosinate (2-amino-4-(hydroxymethylphosphinyl)butanoic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) are available in the US (Johnson et al. 2016; Reddy and Nandula 2012). The development and commercialization of herbicide-tolerant soybeans enabled safe, effective and convenient weed management. However, careful management of these biotechnology crop systems is important for their long-term use. In addition, public acceptance of agricultural biotechnology varies around the globe.

12.5 Expanding Genetic Diversity as a Strategy to Increase Seed Yield

Genetic diversity is an absolute requirement for progress in plant breeding but ironically it is also the greatest barrier to breeding progress. The advantages and disadvantages of genetic diversity depend on the nature of the diversity and our ability to

discern and capture advantageous traits that bring value, apart from the deleterious traits found in diverse germplasm. Historically, the tools and technologies to capture and exploit the value of soybean genetic diversity have been limited. As a result, there have been very few additions to the commercially-used germplasm in the US over the past 50 years and there are large reservoirs of genetic diversity that are yet to be exploited.

Soybean is a domesticated species so, by definition, the entire species is a product of human intervention and thus improved, but primitive cultivars will have lower seed yield and more undesirable agronomic traits compared to commercial cultivars. Within all germplasm collections, primitive cultivars represent the largest number of accessions available so they are very important sources of genetic diversity that should not be ignored.

12.5.1 Breeding History

Scientific soybean breeding began in both China and the US in the 1930s (Bernard et al. 1988; Cui et al. 1999). Although the ancestral lines of these two oldest soybean breeding programs came from the same regions at approximately the same time, they are genetically distinct (Li et al. 2001). For nearly 90 years, breeding programs in these two countries have been selecting for improved cultivars adapted to similar latitudes using divergent gene pools. Japan has been releasing cultivars since 1950 but has only a small soybean breeding effort (Zhou et al. 2000). The first South Korean cultivar developed from intentional hybridization was released in 1969 (Lee et al. 2015b). These Asian countries of ancient soybean cultivation have the potential to supply unique genetic diversity to US soybean breeding programs.

Research to expand the genetic base for US soybean breeding began in the 1960s. Hartwig (1972) crossed selected accessions with the cv. Hill and found no lines equal to the best parent. Thorne and Fehr (1970) and Schoener and Fehr (1979) increased genetic variation for yield in populations that combined exotic and domestic germplasm but the highest yields were always obtained in the populations with no or reduced levels of exotic germplasm. These studies showed no short-term advantage for incorporating exotic germplasm and, regrettably, were not continued to determine if there was a long-term benefit to segregating beneficial genes from deleterious genes derived from the exotic germplasm.

Only a few exotic lines have contributed to the commercially-used gene pool since 1970. In the early 1980s, the Northrup-King Company released S1346, which was derived from a cross with Plant Introduction (PI) 257435. S1346 was widely used as a parent and Sneller (1994, 2003), using pedigree data, determined that it is a major factor in explaining clustering of cultivars from the northern US. PI 91110-1 contributed 25% to the pedigree of IVR 1120, which is the parent of several cultivars developed by commercial companies (http://www.soybase.org/). PI 71506 is a grandparent of cultivars Ripley (MG IV) and Hutcheson (MG V). Hutcheson, released in 1987, was one of the most successful publicly developed cultivars of that

era and is the direct parent of more than 10 cultivars (http://www.soybase.org/). The actual genetic contribution that each of the exotic parents made to these successful cultivars was never determined and that may be why they did not serve as positive examples or provide an incentive for additional use of exotic parents.

12.5.2 Modern Breeding

Examination of pedigree records by Gizlice et al. (1994) determined that over 95% of the genes in public cultivars released by 1988 came from 35 ancestral lines and that genetic base was over 90% established by 1970. Although the US ancestral base was selected from relatively few alternatives (Carter et al. 2004), it has served the breeding community very well. There are several possible explanations for this phenomenon. Studies using molecular markers found the major ancestral lines are at least as, if not more, diverse than selected accessions from the USDA Soybean Germplasm Collection (Brown-Guedira et al. 2000; Kisha et al. 1998; Thompson and Nelson 1998a).

Thompson and Nelson (1998a) examined the pedigrees of publicly developed cultivars and determined that 70% of the gene pool described by Gizlice et al. (1994) came from just seven crosses. In every one of these crosses the parents were from different genetic clusters as defined by Brown-Guedira et al. (2000) and Thompson and Nelson (1998a). The genetic differences between the parents may have been one reason that these were the successful crosses, but almost all the major ancestors made their contribution through only one cross which greatly limited their contribution to the resulting gene pool.

It is also possible that soybean breeders are continually creating new variation and that the current cultivars have variation that was not derived from the original parents. The research of Rasmusson and Phillips (1997) makes a strong case for this in malting barley breeding. The cv. Excel, released in 1990, exceeded the yield of its best parent by 6% and had improvements in malt extract and alpha amylase as well as other important characteristics. The estimated coefficient of parentage of Excel's parents is 0.87. Mating two lines this closely-related would not be expected to produce these gains. They suggest that intergenic recombination, unequal crossing over, transposable elements, DNA methylation, and gene amplification could all be factors that generate variation de novo. More recently, our knowledge of presence/absence variation (McHale et al. 2012; Santos et al. 2016; Zhang et al. 2010), copy number variation (Cook et al. 2012; McHale et al. 2012) and the fluidity of gene copy numbers (Lee et al. 2015b) provide evidence for other means of creating new variation in soybeans. Although we currently have no specific evidence that any of these mechanisms are creating useful genetic variation that is affecting seed yield, it is certainly plausible that it could be occurring. Over the long term, capturing spontaneous changes can be advantageous, but it is a risky strategy to depend on these unpredictable changes, especially as more soybean breeding is based on key molecular markers and less phenotypic selection.

By testing US cultivars in maturity groups II to IV that were released over an 80-year period, Rincker et al. (2014) showed that soybean yield progress has been slow. During this time, seed yields significantly increased, and lodging scores and plant height significantly decreased. Although they demonstrated that soybean breeders are still making progress, their data also showed that the rate of gain has not changed in the past 50 years despite enormous changes in soybean breeding. During this period, the number of soybean breeders in the US has increased, and field and laboratory technology has allowed a great increase in the size of each breeding program, especially within commercial companies. Extensive use of winter nurseries to quickly develop inbred lines, conduct year-round testing and seed increases also has significantly shortened the time between the initial cross and cultivar release. The one aspect of plant breeding that has changed very little is the gene pool used to develop new cultivars. Bringing in new diversity seems like a very reasonable strategy to improve the rate of gain for yield.

Plant breeders access new genetic diversity from ex situ collections. The USDA Soybean Germplasm Collection (http://www.ars-grin.gov/npgs/) is the primary source of *Glycine* germplasm for the world, distributing more than 32,000 seed lots per year over the past decade with approximately 80% of those requests coming from US scientists. Few of the accessions distributed are used as parents and almost all those used as parents have been selected for traits that improve disease or pest resistance or traits other than seed yield.

12.5.3 Exotic Germplasm Sources

There is a common perception that exotic germplasm carries a very large genetic load and identifying high-yielding lines from a cross with an exotic parent is nearly impossible. This perception has some basis in fact and was reinforced by early research that generally used only a few primitive cultivars as the exotic parents. From a plant breeding perspective, exotic is defined as anything that is not domestic. Using this definition there are four major categories of exotic germplasm for soybean breeding: foreign released cultivars, primitive cultivars, wild soybean and perennial *Glycine*. There is currently no compelling data to show that these classes of germplasm have potential to improve the yield of US cultivars.

Recent advances have contributed to improved approaches and better outcomes from exploring more diverse germplasm for soybean breeding. LG90-2550 was released in 1997 (Thompson et al. 1999). It was derived from crossing two experimental lines that each had the pedigree of PI 68658 x Lawrence. LG90-2550 yielded 457 kg/ha more than the Lawrence (Thompson and Nelson 1998b). PI 68658 was introduced from China in 1926, which predated scientific plant breeding. The first confirmed, positive yield QTL that consistently increased yield across years and locations was derived from the primitive cv. PI 68658 (Fox et al. 2015).

LG04-6000 (Nelson and Johnson 2012) was released in 2008 and has PI 436682, the Chinese cv. Jilin 15 released in 1979 by the Jilin Academy of Agricultural

Sciences, in its pedigree. LG04-6000 was tested in 2007 and 2008 in the USDA Northern Uniform IV Test. Averaged over both years it was 296 kg/ha (4.5 bu/ac) higher than the best check in the test (Abney and Crochet, 2007, 2008). More recently LG11-6760 was tested at 18 locations in the USDA Northern Uniform IV test in 2014 and 2015 and averaged 396 kg/ha (6 bu/ac) more than the best check (Schlueter and Scofield 2014; Scofield et al. 2015). Half of the pedigree of LG11-6760 came from PI 561319A and PI 574477. PI 561319A was introduced from China in 1990. It has a name that indicates that it is a primitive cultivar, but two sublines were extracted from the original seed lot, so its origin is unclear. PI 574477 is Fen dou 31 released in Shanxi province in 1990 (Cui et al. 1999). In a recently completed nested association mapping study involving 40 parents crossed to one hub parent (Diers et al. 2018), LG04-6000 and the one of the parents of LG11-6760 (LG00-3372) were found to have QTL associated with high yield that did not exist in any of the 17 high yielding cultivars and experimental lines selected to represent the current gene pool for soybean breeding in most of the northern US. This is the most definitive evidence that there are genes or alleles affecting high yield in exotic germplasm that do not exist in the current commercially use gene pool.

The primary gene pool (Harlan and deWet 1971) of soybean includes the wild soybean so it is not a problem to produce fertile progeny with this interspecific cross; however, it is a major challenge to produce progeny with the yield potential that is equivalent to the soybean parent. The viny plant type, the very small seed size and the extreme pod shattering are all major differences between commercial and wild soybeans (Figs. 12.12a, b, c). Removing these traits from the segregating populations is no small task. The incentive for this challenge is that all measures of genetic diversity indicate that the wild soybean is much more genetically diverse than commercial soybeans (Hyten et al. 2006; Lam et al. 2010; Li and Nelson 2002). These data support the idea that the wild soybean can supply not only novel alleles but also unique genes that are not currently available in domesticated germplasm. Concibido et al. (2003) mapped a yield QTL on chromosome 14 from PI 407305 (*Glycine soja*) in a BC_2 population. When they attempted to confirm this QTL in several elite soybean backgrounds, the positive effect of the QTL was recorded in only 2 of 6 populations. These results indicate that this positive allele from wild soybean may already be in the commercially-used gene pool. Li et al. (2008) using a BC_2 population with PI 245331 (*Glycine soja*) as the donor parent also mapped a yield QTL from the wild soybean parent on chromosome 5. Unfortunately, this QTL was not confirmed, but it mapped to a location within 1 cM of a QTL previously mapped in soybean by Kabelka et al. (2004).

Akpertey et al. (2014) identified BC_2 experimental lines with cv. Williams 82 as the recurrent parent and 4 wild soybean donor parents that were not significantly different in yield from the recurrent parent. Based on genotypic data using 1536 single nucleotide polymorphism (SNP) markers from the Universal Soy Linkage Panel 1.0 (Hyten et al. 2010a), the genetic contribution of the wild soybean parents ranged from 6–30%. Perhaps the most promising results to date come from the report of two experimental lines derived from the cross of the Williams 82 x PI 479752 (*Glycine soja*) that both exceeded the yield of the soybean parent (Swarm

Fig. 12.12 Wild soybean accessions. (**a**) Accession PI441001 *Glycine tomentella* showing moderate viny morphology, (**b**) Accession PI468400B *Glycine soja* showing extremely viny morphology, **c** Diversity of soybean seed types from cultivated, exotic and wild soybeans. (Photo courtesy of E. Peregrine and R.L. Nelson)

2017). One line was the same maturity as Williams 82 and the other matured 2 weeks earlier. There is no data yet on the loci associated with this yield increase or if they are unique to wild soybean, but this first report of genes from wild soybean that can increase the yield of a soybean cultivar is an important research milestone.

There are 26 perennial *Glycine* species in the soybean tertiary gene pool, which means that it takes extraordinary measures to produce fertile progeny (Harlan and

deWet 1971). Because of this difficulty, there has been the least amount of breeding done with the perennial *Glycine* species. To date, fertile progeny have been tested from crosses involving only one accession of *G. tomentella* accession PI441001 (Fig. 12.12a) and the soybean cv. Dwight (Singh and Nelson 2014, 2015). Akpertey et al. (2018) identified a BC_4 line with the same maturity as Dwight that yielded 242 kg/ha (3.6 bu/ac) more than Dwight. From the same experiment, a BC_3 line was 6 days later than Dwight and 477 kg/ha (7.1 bu/ac) higher yielding. Finding yield increases of this magnitude was unexpected. This initial research with one accession from one perennial species is very promising and has enormous potential for changing cultivar development strategies.

12.6 Soybean Mutation Breeding

Genetic variation is the source and basis for plant breeding and crop improvement. In the past, natural variation or spontaneous mutations were the only source of novel genetic diversity that plant breeders could exploit in selecting plants for domestication and breeding (Shu et al. 2012). As described elsewhere in this chapter, several economically-important crops including soybean possess a narrow genetic base, thus limiting efforts for trait discovery and genomic improvements. Furthermore, breeding and intensive selection focusing on enhanced agronomic performance have created exceptionally uniform modern soybean cultivars and a narrow genetic background (Campbell and Stupar 2016; Hyten et al. 2006). Therefore, increasing soybean genetic variation is essential to achieve sustained yield gains as well as resources for trait discovery. There are several potential strategies that can be used to introduce novel variation into the soybean germplasm, including modern tools of biotechnology such as genetic transformation and genome editing. These serve as powerful alternatives for creating genetic variation that complement efforts to cross modern soybean lines with wild or exotic relatives. Biotechnology and wild introgression strategies can be very powerful methods for introducing useful genetic variation but may not be appropriate for some projects. In some cases, random mutagenesis using chemical or irradiation treatments is an attractive approach for increasing variation and identifying useful traits. Furthermore, mutagenesis is a classical strategy and populations developed with these methods do not require time-consuming genetic crosses or tissue culture propagation. The mutagenized materials can he grown, handled and transferred without legal or regulatory restrictions. Some mutants that emerge from a mutation pipeline may carry novel and transmissible traits that are favorable and/or desirable. These traits can be used for mutation breeding, a process in which the desirable traits are introgressed into elite germplasm for cultivar development. This section reviews the methodologies that underlie mutation breeding in soybean and identifies examples in which specific traits have been enhanced using this approach.

12.6.1 Conventional Mutagenesis in Soybean

Several different mutagens have been used to generate large soybean mutant populations and create novel mutant alleles (Campbell and Stupar 2016). Moreover, there are examples in which the functions of soybean genes have been elucidated using mutant-based reverse genetic approaches (Lakhssassi et al. 2017b; Xia et al. 2012). The efficacy of introducing mutations in a given plant depends on several factors, including: the mutagenized tissue type (e.g. seeds or pollen), mutagen type, mutagen dosage, duration of treatment and rate of plant survival following the treatment. In the majority of soybean mutation experiments, mature seeds have been successfully treated to obtain large mutant populations. Seeds are effective for this type of work because they are intuitive to use, easy to handle, and can survive damaging treatments. The two major classes of plant mutagens consist of irradiation by fast neutron, gamma radiation, X-rays and ion beam as well as chemical mutagens including ethylmethane sulphonate (EMS), N-nitroso-N-methylurea (NMU) and ethyl nitrosourea (ENU) treatments. Both classes have been successfully used to develop numerous soybean mutant populations (Akao and Kouchi 1992; Arase et al. 2011; Bolon et al. 2011; Cooper et al. 2008; Pavadai et al. 2010). Each mutagen treatment introduces a signature footprint of allelic variation in the genome, providing several choices for specific goals and follow-up experiments. In conventional mutagenesis, researchers oftentimes run small pilot experiments to test different mutagen dosages and durations, seeking to identify and optimize a protocol that generates substantial genetic variation without destroying the seeds. Many researchers measure the survival rate of their treated seed and seek to find the dosage and duration that produces a survival rate between ~ 40–80%. Once these parameters have been identified, the research can proceed to develop a large population using these mutagenesis conditions. EMS, ENU and NMU are examples of chemical mutagens that have been used in soybean. EMS is the most commonly used mutagen in plants due to its high mutation rate, cost-effectiveness, chemical availability and ease of handling (Table 12.1). These chemical mutagens introduce single base substitutions (most often nucleotide transitions) at high density, which results in non-sense, mis-sense and mis-splicing mutations that can lead to an alteration or elimination of gene function (Anai 2012). The induced mutations are essentially distributed at random throughout the genome. As evident from several studies, point mutations are less detrimental to plant development than large deletions (or rearrangements), hence a high degree of saturation can be achieved in chemical mutant populations, which facilitates investigations of gene function on a genomic level (Gilchrist and Haughn 2010). Moreover, the protocols for soybean EMS and NMU mutagenesis are well established and highly reproducible (Cooper et al. 2008). In soybean, the EMS concentration typically ranges from 0.35–0.70% (v/v), while ENU and NMU range from 2–4.5 mM. These levels have been successfully used in several studies (Cooper et al. 2008; Lakhssassi et al. 2017b; Watanabe et al. 2009). A mutation frequency of 1/140 kb (Cooper et al. 2008) and 1/769 kb (Anai 2012) have been reported in soybean. However, a mutant population developed by repeated

Table 12.1 List of soybean traits improved using chemical and irradiation mutagenesis

Trait	Mutagen	References
Seed fatty acids (oleic, linolenic, stearic, palmitic)	EMS, NMU, GR	Anai (2012), Bubeck et al. (1989), Carrero-Colón et al. (2014), Dierking and Bilyeu (2009a), Fehr (2007), Gillman et al. (2014), Lakhssassi et al. (2017a, b), and Takagi (1998)
Sucrose content (KASI)	FN	Dobbels et al. (2017)
Oil and protein	EMS, GR	Bolon et al. (2011, 2014), Espina et al. (2018), Hudson (2012), and Tsuda et al. (2015)
Lipoxygenase, lectin	GR	George (2006) and Hajika et al. (1991)
Raffinose synthase (RS2)	EMS	Dierking and Bilyeu (2009b)
Phytic acid	EMS	Wilcox et al. (2000) and Yuan et al. (2007)
Nodulation	EMS, GR	Akao and Kouchi (1992), Carroll et al. (1985), Gremaud and Harper (1989), Lakhssassi et al. (2017a, b), Men et al. (2002), and Takahashi et al. (2005)
Chlorophyll deficiency	FN, GR	Arase et al. (2011) and Karthika and Lakshmi (2006)
Soybean cyst nematode	EMS	Liu et al. (2012b) and Shiming et al. (2017)
Flowering (maturity)	EMS and X-ray	Watanabe et al. (2009) and Xia et al. (2012)
Herbicide tolerance	ENU, NMU	Sebastian and Chaleff (1987)
Trichrome	FN	Campbell et al. (2016)
Short Petiole	FN	Bolon et al. (2011, 2014)
Four seed pod	EMS	Zhu and Sun (2006)
Morphology and architecture	FN, EMS	Bolon et al. (2011); Espina et al. (2018); Hwang et al. (2015)

EMS Ethylmethane sulphonate , *NMU* N-nitroso-N-methylurea, *ENU* Ethyl nitrosourea, *GR* Gamma ray, *FN* Fast neutron

EMS treatment of soybean achieved relatively higher mutation frequencies of 1/11.8 kb and 1/74 kb in soybean (Li et al. 2017b; Tsuda et al. 2015).

Irradiation mutagens such as gamma radiation (GR) and fast neutrons (FN) are a particularly promising source of mutagenesis due to the potential to create deletions in a wide range of sizes for gene knockouts and disruptions (Bolon et al. 2011). The FN not only creates small (few base pairs) or large (several megabases) deletions in the plant genome but it may induce segment duplications, translocations and inversions (Bolon et al. 2014). The most successful dosage rates reported in soybean range from 16–32 Gy for FN, 100-200 Gy for X-rays and 100–500 Gy for GR (Alikamanoglu et al. 2011; Anai 2012; Atak et al. 2004; Bolon et al. 2011). The larger deletions most often generate detrimental effects. In recent years, ion beam radiation has been used in mutation breeding because the beams have been shown to cause more severe breaks in DNA than the traditional sources of radiation like GR and X-rays (Arase et al. 2011; Yamaguchi 2018). In irradiated populations, the frequency of mutations is lower than chemical mutant populations, however the nature of a single lesion can have a high impact (i.e. a single large deletion in an

irradiated mutant can knock out hundreds of genes, whereas a chemically-induced single point mutation will likely impact only one gene). Two large FN populations using line M92-220 and cv. Williams 82 are publicly available (https://soybase.org/mutants/).

12.6.2 Molecular Analysis

Several soybean mutant populations have been created in recent decades, and many have been deeply screened (thousands of plants) by forward genetics, particularly for morphological and seed composition traits (Bolon et al. 2014; Espina et al. 2018; Tsuda et al. 2015). Some populations also have been screened for molecular allelic variation; however, such screens have tended to examine either many individuals for very few genes, or very few individuals for many genes (Anai 2012; Cooper et al. 2008; Watanabe et al. 2009). Methods for screening many individuals for few genes include the Deletagene method (Li et al. 2002), TILLING (targeting induced local lesions in genomes) (Cooper et al. 2008), high-resolution melting (Gady et al. 2009) and KeyPoint technology (Rigola 2009). Among these, TILLING has been a particularly useful strategy in soybean and has been used to identify genes involved in flowering and maturity loci (Watanabe et al. 2009), SCN resistance (Liu et al. 2012b), and seed composition traits (Dierking and Bilyeu 2009a; Gillman et al. 2013; Lakhssassi et al. 2017a). Screening of many genes for few individuals can be accomplished with comparative genome hybridization (CGH) (Bolon et al. 2011) or next-generation sequencing (NGS) platforms (Tsuda et al. 2015).

While the methods mentioned above can be used (and have been used) to advance soybean mutation breeding objectives, it seems that resources and platforms that provide the ability to screen many genes among many individuals would be essential to make mutation breeding more effective (and more utilized) for soybean genetic improvement in the future. Molecular analyses of many genes in many individuals requires substantial and expensive NGS (next generation sequencing) efforts, but has been recently achieved by other crop species communities (Krasileva et al. 2017; Li et al. 2017a). However, no such resource has been fully developed for soybean to date. It is conceivable that a NGS pipeline could be developed to identify nearly any sequence variation in a large mutant population at very high precision. This would include point mutations, large and small (e.g. single-base) deletions, gene copy number alteration, and more complex rearrangements (e.g. chromosomal translocations). A database that comprehensively catalogs sequence variants in a mutant population would provide a foundational resource for soybean reverse genetics and trait discovery. Once valuable mutations are identified, allele-specific markers and genotyping assays could be developed for subsequent marker-assisted selection (Patil et al. 2017). However, questions arise as to which genotyping technologies may best discover *all* genetic variants in the genome, while considering

factors such as throughput, cost effectiveness, reproducibility and ease of downstream analysis.

12.6.3 Enhanced Traits and Improved Cultivars

The mutation breeding approach has aided breeders in the development of useful genetic resources for improving various traits in soybean (Table 12.1). In some crop species, mutation breeding has led to adoption of traits and new cultivars that became highly prevalent in the market (Ahloowalia et al. 2004). There have also been cultivars released from mutation breeding pipelines in soybean, in different parts of the world (Shu and Manjaya 2007; Talukdar 2014). From 1960–2017, over 200 improved soybean cultivars developed using physical or chemical mutagenesis were officially registered to the Mutant Cultivars Database (FAO/IAEA; https://nucleus.iaea.org/Pages/mvd.aspx). The majority of these mutant cultivars are being grown in China, Japan, India, South Korea and Vietnam, and were selected for high yield, early maturity, disease resistance or high protein content (Talukdar 2014). In Vietnam, nearly 50% of the improved soybean cultivars grown are derived from induced mutations (Vinh et al. 2009). These cultivars include DT-17, -18 for abiotic stress tolerance, DT-10, -11, -13, -84, -2008 for high yield and disease resistance, and VND95-20 and TNDB-100 for early maturity and high yield. Similarly, cvs. Heinong-26, Raiden, Beinong-103, Raikou, Kosuzu, Josaengseori and Tiefeng are grown as high yielding cultivars in China, South Korea, and Japan (Ahloowalia et al. 2004; Ha et al. 2014; Shu and Manjaya 2007; Talukdar 2014). Takahashi et al. (2005) developed a supernodulating cv. Sakukei-4 with improved plant vigor and yield. Himso-1563 and TS-82 mutant cultivars of soybean possess high protein and low fiber content, and were developed using GR and EMS, respectively (Kavithamani et al. 2010). The mutant cultivars MACS-11, MACS-107, NRC-2 (mutant of cv. Bragg) and NRC-07 are widely grown in several parts of India as a source of disease resistance and high yield (Shu and Manjaya 2007; Talukdar 2014).

In the US and other parts of the world, several improved soybean cultivars were developed by inducing mutations directly into well-adapted cultivars or introgressing induced mutation into well-adapted genetic backgrounds. The major focus has been to improve seed composition traits (fatty acids, lipoxygenase, oligosaccharides, phytic acid), disease resistance, abiotic stress tolerance and yield (Espina et al. 2018; Shiming et al. 2017). The cvs. IA3017, IA3024, IA2064 and RG10 (C1640) with low linolenic acid were developed and commercialized by Iowa State University (Fehr 2007). Development of soybean cultivars with high oleic and low linolenic acid (HOLL) is a priority for the soybean oil industry, as it may improve oil shelf-life and have health benefits for humans. Similarly, development of soybean cultivars with improved digestibility is a priority for the feed industry, as it may provide health benefits for animals. Several research programs are currently working to combine natural and induced mutants to develop these traits in suitable maturity groups. Using conventional breeding and the advent of MAS, several cul-

tivars with HOLL have been developed and/or are in advanced testing stages (Bachleda et al. 2017). Gamma irradiation was used to mutate three lipoxygenase genes (*L1-3*) to reduce the beany or grassy flavor of soybean seed products (thereby making it more desirable for human food). This resulted in the development of cvs. IA2017, IA2029, IA2032, Ichihime and OX756 (Ahloowalia et al. 2004; Hajika et al. 1991). In addition to seed composition traits, soybean researchers are also interested in creating novel mutant cultivars for improved yield, targeting traits such as photosynthetic activity, shoot architecture, slow-wilting and root biomass. Soybean mutant cvs. Hector, Centauro, Bajio Plus, Esperanza and Parana were released for cultivation in Mexico and Brazil, and were selected for reduced dehiscence, increased height to first pod and reduced lodging (Talukdar 2014; Tulmann Neto and Alves 1997).

Beyond direct cultivar development, there are many examples of successful studies that utilized classical mutagenesis to investigate gene functions in soybean. Mutations for important agronomic traits, including nodulation (Men et al. 2002, Takahashi et al. 2005), fatty acid profile (Dierking and Bilyeu 2009b; Reinprecht et al. 2009), flowering (Watanabe et al. 2009), soybean cyst nematode (Liu et al. 2012b), stearic acid (Gillman et al. 2014; Lakhssassi et al. 2017b), sucrose (Dobbels et al. 2017), male-sterility (Frasch et al. 2010) and others, have been discovered using the principles of mutation breeding and functional genomics. For example, Liu et al. (2012b) used TILLING to identify missense mutations in a gene *GmSHMT08* at the Rhg4 locus in the background Forrest cv.; these mutations were used to confirm the function of *GmSHMT08* in SCN resistance. Watanabe et al. (2009) identified an EMS-induced mutation in a phytochrome A gene (*E3*) associated with soybean maturity. Dobbels et al. (2017) discovered that an FN-induced chromosomal translocation disrupted an oil biosynthesis related gene (*KASI*) and elevated soybean seed sucrose content up to 8.1%.

12.7 Molecular Breeding: Techniques and Tools of Soybean Biotechnology

Soybean yields, like all commodity crops, are governed by two general components: the agronomic inputs implemented over the growing season and the underlying genetics of the seed sown. Precision technologies for targeted nutrient, water and pesticide application are approaches that are translating to reduced inputs, which in turn lead to larger profit margins for producers. On the genetics side, a repertoire of biotechnology tools (Benning and Sweetlove 2016; Liu and Stewart 2015) has become available over the past few decades. These tools hold great potential to complement soybean breeding programs to add novel bits of genetic variation into the crop for targeted input and output traits. A prerequisite to exploit these tools is the ability to clone, characterize, manipulate and deliver DNA into soybean cells in germinal lineages to permit inheritance of the new trait. This prerequisite was

demonstrated in the mid 1980s with reports communicating the production of the first transgenic soybean plants (Hinchee et al. 1988; McCabe et al. 1988). These developments translated to the marketplace, providing additional weed management strategies for producers (Behrens et al. 2007; Padgette et al. 1995), along with improved oil for direct consumer applications (Kinney and Knowlton 1997).

12.7.1 Enhanced Input Traits and Defensive Traits

The Roundup Ready® glyphosate tolerant soybean was one of the first engineered crops to reach the market, and today accounts for fully half of all the biotech crop hectarage. In 2018, almost 80% of all soybeans planted on earth were glyphosate tolerant, with the US, Brazil, Argentina, Paraguay, South Africa, Bolivia and Uruguay having over 90% adoption rates (ISAAA 2018). Anecdotally, some countries that are GMO-free, such as Ukraine, have equally high adoption rates of Roundup Ready soybean.

The advent of Roundup Ready soybean has made it easier to use no-till agricultural practices. Not only do these reduce soil erosion and enhance soil health, tilling 0.4 ha (1 acre) requires the same amount of fuel as driving a family car for 225 km (140 miles), resulting in additional fuel savings. During this period, farmers around the world also increased their income by USD 54.5 billion simply by adopting Roundup Ready soybean (Brookes and Barfoot 2018a,b).

The adoption of Roundup Ready soybean led to a substitution of older herbicides with glyphosate, resulting in a net increase of 0.4% active ingredient applied between 1996–2016 on a global basis. However, the environmental impact, as measure by the Environmental Impact Quotient, actually decreased 13.4% during this same period (Brookes and Barfoot 2018a).

Such a widespread use of glyphosate has inevitably resulted in the evolution of glyphosate tolerant weeds, making the use of additional herbicides necessary. Specifically, 14% of USA farmers used a second herbicide with glyphosate in 2006. Ten years later, the figure has grown to 89% (Brookes and Barfoot 2018a). Therefore, crop protection and soybean seed companies have scrambled to develop and commercialize other herbicide tolerant soybeans, as determined from notifications for field testing or petitions for deregulation. These include resistance to glufosinate (Liberty Link®), 2,4-D (Enlist®), Dicamba (Xtend®), isoxaflutole (Balance Bean®), and mesotrione (the M in MGI®).

Insect resistant soybean, obtained by transformation with a *Bacillus thuringiensis,* Bt, gene, was not deployed until 2013, and sold as a stacked trait combined with herbicide tolerance. Even today, it is only planted in South America, where the ability to mitigate losses from insects led to an additional 13.4 million mt of harvested soybean (Brookes and Barfoot 2018b). Additional soybeans with pyramided Bt genes are making their way through the regulatory process. These are expected to be more durable than single genes in delaying insect resistance. More options exist

to enhance the durability of Bt soybean, including pyramiding the transgenes with conventionally bred insect resistance genes (Ortega et al. 2016).

As the knowledge of basic plant biochemistry and metabolism increases, it will be possible to alter physiological traits that may enhance yield. For example, engineering soybean with ictB, a cyanobacterial membrane protein (Lieman-Hurwitz et al. 2003), led to increase in the crop's photosynthetic assimilation capacity (Hay et al. 2017), while outcomes from studies into carbon/nitrogen partitioning in seeds of the model plant *Arabidopsis thaliana* translated to improvements in total protein levels in soybean, without compromise in oil or productivity (Li et al. 2015a).

12.7.2 Output Traits

In regard to output traits that directly impact the consumer, high oleic soybean (Kinney and Knowlton 1997) is reaching the marketplace. Ever since the Food and Drug Administration removed GRAS status from transfats (created when conventional soybean oil is hydrogenated) the United Soybean Board has moved aggressively to replace 6.5M ha (16M ac) of the US commodity soybean crop with high oleic cultivars. The development of high oleic soybean was possible due to advancements in both RNA interference (RNAi) (Buhr et al. 2002; Kinney and Knowlton 1997) and chemical mutagenesis (Pham et al. 2010).

In addition to protein quantity, quality is an active area of biotechnology research in soybean. Perhaps in the future, improved protein quality will be obtained by selectively permitting the expression of proteins with higher content of essential amino acids (Schmidt et al. 2011) or by bypassing the cell's feedback mechanisms (Yu et al. 2018). Likewise, the development of seed low in phytic acid could increase phosphorus availability in livestock feed (Punjabi et al. 2018). Other desirable and market-specific output traits include enhanced nutritional and health value for soybean as a food and feed source (Fig. 12.13).

Gene-silencing by RNAi has other applications as well, such as to provide strategies for protection of yield via virus resistance (Kumari et al. 2018; Yang et al. 2017b; Zhang et al. 2011). A variant of RNAi, phasi-RNA, is also effective for trait silencing, and has the added advantage in soybean that hairpin constructs are not needed to trigger silencing (Jacobs et al. 2015b). Hairpin constructs can be unstable, and thus difficult to work with, so their avoidance facilitates the use of silencing.

As discussed elsewhere in this chapter, abiotic stress and disease resistance are areas that particularly lend themselves to biotechnology applications, and there are numerous examples. Just to cite the most recent examples, different genes are reported to enhance resistance to osmotic stress (Qin et al. 2017; Wang et al. 2017, 2018b; Xue et al. 2018), *Phytophthora* (Du et al. 2018), cyst nematodes (Yang et al. 2017a), and *Fusarium* (Wang et al. 2018a). Notably, these are stressors that are otherwise difficult to control with conventional breeding.

Fig. 12.13 Soybean with genetically engineered enhanced output traits (orange soy). Immature soybean pod (top) carrying an 8 transgene stack designed to simultaneously accumulate key aquaculture feed ingredients, including the keto-carotenoid astaxanthin, very long chain omega-s fatty acids and high levels of tocotrienol; wild-type immature soybean (bottom). (Photo courtesy of T.E. Clemente)

12.7.3 Biotechnology for New Uses

Moving away from traditional agriculture, engineered soybean seeds can be used as biofactories due to their ability to accumulate and stably store high levels of protein. For example, fully functional human epidermal growth factor has been produced in soybean (He and Schmidt 2016). Production of such heterologous proteins can be facilitated if the main storage proteins are removed first (Schmidt and Herman 2008).

The current traits on the market in soybean are derived from the first wave of single gene traits obtained via transgenes. Looking ahead, the development of gene synthesis technologies (Kosuri and Church 2014) will greatly aid the introduction gene stacks to allow for the assembly of metabolic pathways that lead to the production of high value compounds with food, feed, or other applications (Eckert et al. 2006; Park et al. 2016; Pierce et al. 2015). Such enablement of metabolic engineering/synthetic biology will enable soybean to be repurposed for new uses, including the production of very long-chain omega 3 polyunsaturated fats (Wilkes and Bringe 2015). Additional modifications that remove proteins not tolerated by carnivorous fish can result in soybean meal tailor-made for aquaculture (Herman and Schmidt 2016).

Ketocarotenoids are important dietary supplements for salmon and shrimp farming, and laying hens. These compounds produce the flesh and yolk coloration expected by consumers. Both canthaxanthin (Pierce et al. 2015) for layer hens and

astaxanthin, stacked with omega-3 fatty acid production have been reported in engineered soybean (Fig. 12.13) (Park et al. 2016).

12.7.4 New Technologies

On the academic side, technological advances in gene and vector construction, coupled with the wealth of genomics resources available for soybean, fosters its use as a model species within the Fabaceae (Joshi et al. 2013; Libault et al. 2010; Schmutz et al. 2010; Severin et al. 2010; Song et al. 2013, 2016), which in turn, helps foster future innovations to be translated to the marketplace. This rich pool of resources in soybean genomics and metabolomics, most of which were built primarily from public sector investments (Hossain et al. 2015; Kusano et al. 2015), is now coupled with capacity for high-throughput phenotyping platforms (Bai et al. 2016; Xavier et al. 2017), and tools for transposon mutagenesis (Cui et al. 2013; Hancock et al. 2011; Mathieu et al. 2009) using transposons from maize, rice, or tobacco engineered into soybean are facilitating gene discovery and characterization, and making it possible to link genes with their phenotype.

The tool box for the creation of novel genetic variation in soybean has been further expanded by the advent of genome editing technologies (Belhaj et al. 2013, Hartung and Schiemann 2014). These include transcription activator like effector nucleases (TALENs) (Curtin et al. 2012) that have been effectively employed to recapitulate the high oleic acid soybean oil trait, developed earlier, by creating TALEN-induced null mutations in the delta-12 fatty acid desaturase gene FAD2-1 (Haun et al. 2014). More recently, the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system was used effectively for targeted editing in soybean (Cai et al. 2018; Jacobs et al. 2015a; Li et al. 2015b), and has the added advantage that multiple genes can be edited simultaneously (Kanazashi et al. 2018).

Moreover, with the availability of a robust hairy root system (*Agrobacterium rhizogenes*) in soybean (Collier et al. 2006), a high-throughput means for testing and vetting of CRISPR guide RNA designs to maximize frequency desired edits at the whole plant level is an option for researchers to exploit (Curtin et al. 2018; Jacobs et al. 2015a). More recently, high-throughput screening is also being facilitated through the availability of protoplast transient expression systems (Wu and Hanzawa 2018).

12.8 Genetics, Genomics and Phenomics: From Academic Discovery to Company Commercialization

Current knowledge of soybean genetics along with new genomic and phenomic technologies gives commercial breeders powerful tools to improve soybean production. These tools are aimed at some aspect of the response to selection, often called

the breeder's equation (Heffner et al. 2010; Lush 1943). The following equation is a modified version of the breeder's equation that is practical for commercial breeding:

$R_t = \frac{ir\sigma_A}{y\$}$ where R_t is genetic gain over time, i is selection intensity, r is selection accuracy, σ_A is genetic variance, y is years per cycle, and $ is cost.

$$Genetic\ gain\ over\ time = \frac{selection\ intensity * selection\ accuracy * genetic\ variance}{years\ per\ cycle * cost}$$

Technologies that are successfully deployed into commercial breeding programs target some aspect of this equation while keeping a balance with the other variables to maximize genetic gain over time. For example, if a new method is significantly cheaper, it may enable the breeder to increase genetic variance by testing more lines. Alternatively, if a high-throughput phenotyping method increases selection accuracy but it also increases cost, then the amount of genetic variance is reduced since the higher cost will reduce the number of breeding lines that can be tested.

Technologies deployed to commercial breeding often go through three phases: discovery, development and deployment. The discovery process tests several different technologies with the goal of identifying the technologies with the most potential to improve genetic gain over time. Then, researchers develop these technologies to deploy into breeding programs. This development process requires making technology more robust and creating the ability to run the technology at an economically feasible, larger scale. This process often involves a handoff at some point in the development phase to private industry. Since industry has the monetary resources and lab requirements needed for a new technology, companies can more easily replace their current breeding methodologies. Once the technology is sufficiently developed, it can be deployed on a commercial scale, helping to realize the real impact on crop improvement from the initial academic discovery. In soybean, there have been several examples of genetic and genomic technologies being discovered and developed in the public sector and later used for commercial development of soybean cultivars.

12.8.1 Marker-Assisted Selection (MAS)

A key to enabling breeders to start using molecular technology to assist in selecting lines was the development of *breeder-friendly* marker technology. The first generation of breeder-friendly technology was the development of SSR (Simple Sequence Repeats) markers (Akkaya et al. 1992). These markers were PCR-based markers that targeted simple sequence repeat differences. The development of soybean SSR markers targeting a major resistant gene to soybean cyst nematode (SCN, *Heterodera*

glycines) demonstrated the power of using markers for selection (Cregan et al. 1999). This resistant gene, *rhg1*, engendered resistance to SCN. The development of Satt309 allowed breeders to run this simple PCR marker to select lines that were resistant instead of testing for resistance in the greenhouse. The breeders' ability to select resistance based on the marker significantly increased their selection accuracy since the marker allele has 100% heritability. This also decreased their cost to screen lines, allowing them to run more lines or reinvest those savings into other aspects of the breeding program. Screening more lines increased the genetic variance for other traits, such as yield, among populations that were fixed or significantly enriched for SCN resistance.

The commercial application of these markers was quickly realized and adopted for SCN and many other traits, especially disease resistance. The use of markers in commercial soybean development is evident by a very early patent by Pioneer Hi-bred International that claimed methods to select for *rhg1* along with other SCN resistance genes (Webb 1996). The continued application of MAS in private breeding companies is demonstrated by the number of patents related to methods to select for major genes of interest using MAS (Narvel et al. 2012; Sebastian et al. 2009; Yu et al. 2016a). These patented disclosures, along with the frequent updates to Soybase (www.soybase.org) of QTLs and major gene discoveries, demonstrate an on-going investment in the discovery of new targets for marker development.

Newer patents demonstrate that the commercial marker platform has shifted from SSR markers to SNPs (Single Nucleotide Polymorphisms) (Hamilton et al. 2018; Jenkinson et al. 2014; Klaiber et al. 2013). The evolution of marker technology has further enabled high-throughput, multiplexed, small volume and low-cost MAS-driven soybean breeding. The use of SNP markers for MAS is largely attributed to the abundance of genomic SNPs. In addition, next generation sequencing technology has made it easy to discover SNPs for new marker development (Hyten et al. 2010b). While markers have been very effective in selecting traits with major loci, they have not been effective for selecting for traits that are more complex.

12.8.2 Genomic Selection

The success of implementing MAS for major genetic loci has led to more complex application of genetic markers. Yield is a quantitative trait that has low heritability and has many loci contributing small effects on the yield variations across cultivars. Employing MAS for low heritable traits like yield has significantly less accuracy to justify the additional cost of running the markers and has not been shown to be effective for increasing the rate of yield gain. To increase the genetic gain over time for yield, genomic selection is being developed and deployed by both public and commercial breeders (Sebastian et al. 2012; Xavier et al. 2016).

For genomic selection to be effective, new tools must be developed to decrease the cost of obtaining the marker data needed for the populations where selection will occur. Genotyping-by-sequencing is a promising technology but still needs

development to reduce the cost even further than the current restriction enzyme methods available to breeders (Elshire et al. 2011). Reducing the cost enables breeders to apply genomic selection early in the breeding program when they have little field-derived yield data. While doing genomic selection at the F_2 seed stage would be ideal, the numbers of lines and the cost would be excessive. For most breeding programs, it is more beneficial to apply genomic selection at the point when single rows are derived (Sebastian et al. 2012). This would allow predictions to be made on many lines when no phenotypic yield data are available. This prediction would then be used to enrich the breeding populations for better-yielding lines for subsequent replicated field tests to select for the highest-yielding lines.

12.8.3 Soybean Genome Sequence

One of the most significant resources developed by the public sector is the soybean genome sequence (Schmutz et al. 2010). This sequence is the foundation of most soybean genomics and has enabled significant discovery and development of genomic applications to breeding. The soybean genome is relatively small at 1.1 billion basepairs. It is made up of 20 chromosomes with a genetic map of around 2400 cM (Song et al. 2016).

Williams 82 was the first soybean line to be completely sequenced. A key feature revealed by the whole genome sequence was the large amount of heterochromatin in the pericentromeric regions of soybean. This heterochromatin makes up 60% of the genome (Schmutz et al. 2010). It contains mostly retrotransposons and severely reduces recombination. This reduction in recombination has a large effect on using genomics technologies in breeding. For MAS, any targets being selected in heterochromatin regions could have severe linkage drag associated with them. Fine mapping genes in heterochromatin is also extremely difficult due to the low amount of recombination. A major gene for protein discovered in the heterochromatin of chromosome 20 is one example (Diers et al. 1992). With this gene located in the heterochromatin, fine mapping it and cloning it has remained elusive over the last 26 years, despite significant effort by the public sector (Bolon et al. 2010; Chung et al. 2003; Diers et al. 1992; Nichols et al. 2006; Sebolt et al. 2000).

12.8.4 Soybean Genomic Variation

While most QTLs for MAS have been mapped in biparental populations, genome-wide association studies (GWAS) have become more common for discovery and fine mapping of important loci. GWAS uses unstructured populations and high-density marker data to make marker/trait associations. A key dataset in soybean for GWAS has been the genotyping of the USDA germplasm collection with an Illumina 50K SNP chip (Song et al. 2013, 2015). This germplasm collection holds over

20,000 accessions that are readily available to both public and private researchers. Since the collection has been fingerprinted with the 50K chip, the public community has undertaken a large research effort to phenotype it to make new associations (Bandillo et al. 2017; Schneider et al. 2016; Zhang et al. 2016) . Even though soybean has high linkage disequilibrium, GWAS has better resolution than most QTL mapping studies for identifying the location of the locus associated with the trait. In addition, since the GWAS is performed in diverse unrelated populations, the top SNP marker hits for GWAS are excellent candidates to be converted into markers for MAS. These highly-associated markers have a much higher probability for working across breeding populations. This is a big advantage for commercialization since only one marker is needed across breeding populations that segregate for the trait. Alternatively, if a marker is tightly linked to the locus of interest, but not highly associated, then it will only be polymorphic in a small number of populations that segregate for the trait being selected. This forces breeders to develop multiple markers for a single locus making it less desirable for commercialization.

12.8.5 Phenomics

The increasingly low cost of obtaining genetic information has highlighted the need to be able to obtain phenotypic data more economically. This need of collecting phenotypes is fueling the current work in discovering new phenomics methods. Just as genomics has dramatically increased throughput, it is expected that researches can develop methods to obtain more phenotype data in high-throughput systems.

Several technologies such as drones, multispectral cameras and automated greenhouses are being explored by academia. Digital imagery has been used in soybean to assess canopy coverage and correlate this coverage with yield (Xavier et al. 2017). Digital imagery also has been used to identify genomic loci associated with canopy coverage (Kaler et al. 2018; Xavier et al. 2017). Another method being explored is using automated greenhouses. A Lemnatec 3D greenhouse has been installed at the University of Nebraska-Lincoln and is being used to phenotype soybeans under water-limiting conditions. This greenhouse has four imaging chambers equipped with visible RGB cameras for leaf area and growth rate; fluorescence cameras for chlorophyll fluorescence and quantify senescence area; infrared cameras to measure leaf temperature and water relations relative transpiration rate; and hyperspectral cameras to measure stress and physiological activity (https://ard.unl.edu/phenotyping/nebraska-innovation-campus-greenhouse). Since the greenhouse only holds 672 pots, this type of phenotyping is collecting high-density imaging data and not high-throughput. Several other academic institutions and companies are implementing similar greenhouses. Studying complex traits like drought in a greenhouse setting often does not translate to field research. There is potential to explore the different imaging technologies available in the greenhouse for insights that can narrow down and improve the most promising technologies. The most promising imaging techniques can be explored with larger scale, high-throughput

field methods such as in-field gantry systems and drones equipped with more sophisticated imaging cameras. As these technologies are explored and refined by the academic sector, only those with the most commercial potential to increase the rate of gain over time will be adopted by companies for further development and deployment.

12.9 Breeding Food-Grade Soybeans

12.9.1 Food-Grade Soybean Production and Markets in the US

Food-grade soybeans are the raw materials for soy food products, including tofu, soymilk, miso, natto, soy sprouts and tempeh. At 4% of US soybean hectarage, a small but consistent proportion of soybean production has been devoted to food-grade cultivars. Over 1.2M ha (3M acres) were planted with food-grade cultivars in 2015–2016, with expectations for a continuation of similar hectarage in the coming years (Strategic Marketing Research and Planning, SMR&P 2016). Where commodity soybean cultivars often have a market life of only 1–3 years, a successful food-grade cultivar can remain on the market for upwards of 20 years (e.g. Buzzell et al. 1991; Fehr et al. 1984; St Martin et al. 1996b).

Tofu soybean cultivars account for 34% of the food-grade hectarage, with the remaining hectarage primarily composed of cultivars used for production of soymilk, miso and natto (Table 12.2). The remaining 4% of food-grade hectarage is divided among a number of markets which include soy sprouts, canned soybeans, tempeh, soy sauce, douchi (fermented black soybeans) and edamame. Unlike commodity soybean cultivars where yield is ultimately selected for over other traits (Rincker et al. 2014), each market class of food-grade soybeans possesses a unique set of traits in addition to yield.

Growers report that soybean premiums are the primary rationale for growing food-grade soybeans over commodity soybeans, followed by herbicide resistant weeds, a desire to move away from glyphosate and low input costs (SMR&P 2016). Food-grade soybean cultivars are generally lower yielding than their commodity counterparts, and, resulting from their non-GMO status, they lack herbicide resis-

Table 12.2 Market classes of US produced food-grade soybean in 2016

Food-grade market class	Hectares (Acres)[a]	% of food-grade soybean hectares[a]
Tofu	394,367 (974,466)	34
Soymilk	260,821 (644,480)	22
Miso	259 733 (641,791)	22
Natto	201,181 (498,658)	17
Others	49,1911 (21,549)	4

[a]Data from SMR&P (2016)

tance. Based on 2016 premium rates for food-grade soybeans, growers need yields of food-grade cultivars to be 97% and 89% of herbicide resistant GMO cultivars for large-seeded and small-seeded food-grade types, respectively, to reach break-even profitability (Mayta et al. 2014; SMR&P 2016). Yields need to be 89% and 86% of conventional cultivars to reach break-even profitability. Thus, for production of food-grade seed to be economical, adequate yield is required for cultivars targeting each food-grade market class.

12.9.2 Characteristics of Food-Grade Cultivars

Traits associated with the ideal food-grade seed is dependent not only on the market class, but also on the producer and production method. However, regardless of the market class and method of production, all food-grade cultivars share several traits. They are non-GMO; have a high seed quality lacking mottling, cracking, etc. and, because most soy food products require a soaking step, rapid water uptake is essential (Graef and Specht 1989; Mullin and Xu 2001; SMR&P 2016). Non-uniform, wrinkled, mottled, or broken seed are considered unfavorable. A light or clear hilum color is preferred, with no hilum color bleeding into the seed coat. Hard or *stone* seeds which do not readily absorb water during soaking are detrimental to processing soy foods, especially in fermented products. In addition to these traits, each market class has specific characteristics related to the seed size, shape and chemical composition which are important. The breeding targets for these traits differ for each class and possibly for each producer.

Traits important for food-grade soybean used in tofu and soymilk production include the chemical composition of the soybean, seed size and shape, as well as hilum color. Tofu is a protein gel produced by the addition of a coagulant, often a calcium of magnesium salt, to soymilk (Wilson 1995). Thus, seed protein content and composition are the most important characteristics in this market class. High seed protein content increases tofu yields and generally generates a firmer product (Huang et al. 2014; Poysa and Woodrow 2002). The negative correlation between seed protein content and yield creates a natural economic limit to how much seed protein can be increased. Yet, cultivars such as Vinton 81 and Harovinton, quality standards for the tofu market class of food-grade soybeans, have seed protein contents exceeding 44% on a dry weight basis (Buzzell et al. 1991; Fehr et al. 1984; Poysa et al. 2008). Food-grade soybean cultivars used for tofu and soymilk production generally have protein content ranging from 36–46% on a dry weight basis and yield 5–25% less than their conventional counterparts (Buzzell et al. 1991; Fehr et al. 1984; Helms et al. 2015; Mian et al. 2017; Poysa et al. 2008; St Martin et al. 1996a, b, 2006).

In addition to the seed protein content, protein subunit composition affects tofu texture. The major seed storage proteins in soybean are glycinin (11S) and β-conglycinin (7S). The 11S/7S ratio has been shown to affect tofu texture and yield, though these effects are inconsistent and potentially dependent on soybean

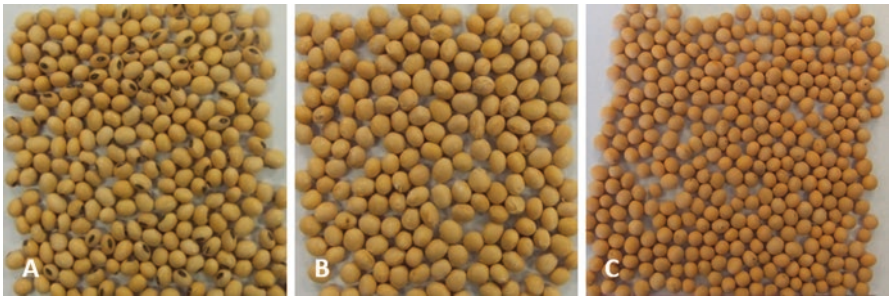

Fig. 12.14 Seed and hilum size, shape and color for commodity soybeans produced in the US for Crush market and processed for oil and meal(**a**), Soymilk and tofu (**b**), and Natto (**c**). (Photos courtesy of Marcia Feller)

cultivar and tofu processing methods (Cai and Chang 1999; Kim and Wicker 2005), with reports of positive (Kang et al. 1991; Mujoo et al. 2003; Murphy et al. 1997), negative (Utsumi and Kinsella 1985) and little to no correlation (Skurray et al. 1980; Taira 1990). In addition, the composition of the subunits themselves can affect tofu quality, with the presence of the 11S A_3 subunit or the absence of the 11S A_4 or the 7S α' subunits increasing tofu firmness (Nishinari et al. 1991; Poysa et al. 2006).

Total sugar content and composition contributes to flavor in soy food products. Soybean is composed primarily of glucose, fructose, sucrose, raffinose and stachyose. Raffinose and stachyose are members of the raffinose family oligosaccharides (RFOs) and are considered to be antinutritionals (Price et al. 1988), and therefore undesirable to be consumed in large quantities. While these products are digested during the fermentation process used in production of miso and natto, they are considered objectionable in unfermented soymilk and tofu (Porter et al. 1990).

Outside of chemical properties of soybean seed, food-grade cultivars targeting tofu or soymilk production are selected for a large (>220 mg/seed), round seed that is preferred by tofu processors (Fig. 12.14). The impact, if any, of the seed size and shape on the resulting tofu or soymilk is not well-defined (Liu 1997). Studies have been inconsistent in determining the effects of seed size and shape on soymilk or tofu yield and tofu quality. While several studies concluded that seed size had no effect on yield or textural qualities of tofu (Cai and Chang 1997; Huang et al. 2014; Lim et al. 1990; Wang and Chang 1995), other studies reported positive correlations between seed size and tofu yield and gel strength (Aziadekey et al. 2002; Bhardwaj et al. 1999; Shih et al. 1997, 2002). Ultimately, selection for traits which are important to the soymilk and tofu producers are crucial for the market success of a food-grade cultivar, regardless of whether the trait(s) affect the yield or textural qualities of the resulting soy food product.

Miso, a fermented soybean paste popular in Japan, is made in a two-step fermentation process in which a cereal or soybean-based starter culture is fermented with soaked and prepared soybeans for a period of several months up to 2 years, with the fermentation time dependent on the desired flavor, fermentation conditions and the

starter culture material (Liu 2008). Medium to large soybean seeds (>120 mg/seed) may be used in the production of miso (Wilson 1995). While the chemical composition of the seed effects the development of flavor in miso, the preferred seed composition is highly dependent on the methods and desired flavor and texture. Therefore, outside of a light hilum color, rapid water-uptake and good agronomic characteristics, there are seldom defined breeding targets in this market class. Often food-grade cultivars developed for the tofu market are used in miso production (e.g. Poysa et al. 2008; Yu et al. 2014).

Natto is a fermented soyfood with a distinctive flavor and sticky texture produced by culturing cooked soybean seeds with bacteria, *Bacillus subtilis* (Watanabe 2006). Consumption of this unique product is primarily in Japan. The main selection criterion for cultivars targeting the natto food-grade market is seed size. Natto is generally produced from soybean seed ≤80 mg/seed. Seed composition contributes to the fermentation, flavor, and texture of natto. Low levels of seed calcium, manganese and boron have been associated with a softer, more favorable natto (Yoshikawa et al. 2014). A high total sugar and sucrose content of soybean seed produces the preferred flavor in natto (Taira 1990).

12.9.3 Characterization and Selection of Food-Grade Traits

Many of the traits important to food-grade cultivar development can be readily evaluated and selected, such as seed size, approximated by 100-seed weight; hilum color, visually assessed; and protein content, measured by near-infrared spectroscopy (NIRS). Perhaps due to the ease of measurement, the genetics of each of these traits has been extensively studied. Seed size is a low to moderately heritable (broad sense heritability, 0.2–0.6), genetically complex trait controlled by numerous loci (www.soybase.org; Cober et al. 1997; Teng et al. 2017; Wu et al. 2018). To date, more than 200 QTLs for seed size, distributed across all 20 chromosomes, have been identified. As discussed elsewhere in this chapter, seed protein is also controlled by numerous loci with over 350 QTL identified (www.soybase.org; Van and McHale 2017). These QTLs include two large and well characterized loci on chromosomes 15 and 20, as well as a number of smaller QTL (Diers et al. 1992). Dependent on the population, heritability of seed protein content is moderate to high. In a panel of food-grade cultivars, heritability was estimated as 0.9 (Stobaugh et al. 2017). In contrast to these complex traits, seed coat and hilum color are relatively simply inherited. Five loci have been identified which control seed coat and hilum color in soybean (*I*, *O*, *R*, *T*, *W1*) (Yang et al. 2010). The *I t R w1* and *I t r* genotypes code for yellow seed coat and hila (Palmer et al. 2004). Germplasm with *l T R w1* and *l T r* genotypes, may have discoloration and are suggested to be called *imperfect yellow* hilum (Cober et al. 1998).

Direct selection for seed size, seed protein and oil content, and hilum color is common in food-grade breeding programs due to the ease in which these traits can be measured. However, the extensive mapping that has been completed also allows

for MAS of these traits. MAS of these traits may be especially useful in backcross breeding programs and where extensive genotyping is already being conducted, such as in combination with genomic selection for yield.

While measurement of protein and oil in soybean seed can be carried out by near infrared spectroscopy (NIRS) in an accurate, rapid, and inexpensive manner, measurement of sugars can be less accurate by NIRS. Soy food processors measure the sugar content and composition of seeds by enzymatic methods or high-performance liquid chromatography (HPLC), both of which can be costly and time-consuming (Giannoccaro et al. 2008; Valliyodan et al. 2015). NIRS calibration methods have been published which have been used in GWAS and QTL analyses for the three major sugars in soybean seed, stachyose, sucrose and raffinose (Poysa and Woodrow 2002; Sato et al. 2012). Breeding lines can initially be selected for specific sugar content and profiles by NIRS. Thus, reducing the number of lines for which total sugar needs to be measured by more intensive methods. Heritabilities of stachyose, sucrose and raffinose seed content are high, approximately 0.7, 0.8 and 0.5, respectively (Jaureguy et al. 2011). Several mutant lines with low raffinose family of oligosaccharides (RFOs) have been characterized with (putative) causal mutations identified in *stachyose synthase* (*STS*) and *raffinose synthase 2* (*Rf2*) for which molecular markers have been designed (Dierking and Bilyeu 2008; Hagely et al. 2013; Patil et al. 2017; Qiu et al. 2015). In addition, over 50 QTLs for seed oligosaccharides and sucrose have been identified. These studies pave the way for MAS of specific carbohydrate profiles.

Soaking of the soybean seed is a critical first step in the production of most soy foods, including tofu, soymilk, miso and natto (Taira 1990). Seeds which fail to take up water are termed hard or *stone* seeds and add significant expense to soy food production. The rapid water uptake required for proper texture for soy foods is a difficult and time-consuming trait to assess, requiring timed weighing of soaked seeds over a 24-hour period (Mullin and Xu 2001). The underlying physiological or chemical cause of hard seeds is not yet clear, though a combination of traits is likely to contribute, including seed coat permeability and potentially calcium content of the seed coat (Mullin and Xu 2001; Zhang et al. 2008). Water uptake and seed hardness are genetically controlled, and several QTLs have been identified for water uptake as well as cooked seed hardness (Keim et al. 1990; Watanabe et al. 2004; Zhang et al. 2008). Further work is needed to fully understand the genetic architecture of seed hardness, but MAS for this trait should be possible.

12.10 Breeding for Seed Composition and Quality Traits: Meal and Oil for Feed and Industrial Uses

Plant breeding can be defined as the improvement of plants to better meet the purposes of humankind, whether those end goals be for aesthetics and enjoyment, human food uses, animal feed, or pharmaceuticals and industrial applications. Plant

breeding goals must satisfy the needs of the producer, processor, and end user as they relate to production quantity, quality, efficiency, and sustainability.

Soybean is the leading vegetable protein and oilseed in global production. Soybeans accounted for 59% of world oilseed production in 2017, with rapeseed in second place at 13% (soystats.com). Soybean protein meal accounts for 70% of the world's vegetable protein meal consumption and 29% of vegetable oil consumption in global markets, with the US the leading producer of soybeans during 2017 (soystats.com). The soybean seed, on average, is comprised of approximately 34% protein, 19% oil, 15% soluble carbohydrates, 15% insoluble carbohydrates, and 4% ash on a 13% moisture basis. Primary uses of soybean meal in the USA are for animal feed, with 55% used in poultry production, 25% in swine production, and the remainder in assorted uses such as beef, dairy and pet food (soystats.com). Soybean protein is the highest quality vegetable protein available on a large scale in global markets. Soy protein is considered a complete protein, containing all essential amino acids in a human diet, with quality and protein digestibility scores comparable to or better than casein, whey, egg white and beef (FAO 1991; Thrane et al. 2017).

Environment can affect soybean seed composition and, along with processing, can influence the quality of the soybean oil and meal products. In general, soybean meal from the US has more consistent quality compared with soybean meal from other sources, even though the US-derived meal may have slightly lower total protein content (Ravindran et al. 2014; Thakur and Hurburgh 2007). The US-derived soybeans and soybean meal were higher in digestibility and the five essential amino acids for poultry and swine feed – lysine, threonine, tryptophan, methionine and cysteine. While there is some genetic variation for amino-acid composition in soybean, there is significant influence of environment, genotype-environment interaction effects, and compensation in the plant for protein balance in seeds (Carrera et al. 2011; Herman 2014; Kinney et al. 2001; Panthee et al. 2006; Pazdernik et al. 1997; Schmidt et al. 2011; Serretti et al. 1994). For the purposes of this section, specific amino acid modifications are not considered, but we will focus mainly on breeding to achieve appropriate balance of seed protein, oil and carbohydrates to meet processor and end-user needs as it relates to general-use, commodity soybean, while improving yield to meet producer needs and growing global demand.

To be effective in breeding, it is important to clearly define goals and to understand relationships among the target traits, environmental effects and genotype-environment interactions. Seed protein concentration and seed oil concentration generally exhibit relatively high heritability of 0.6–0.9 for inbred lines on an entry mean basis, though lower heritability values have been reported in some populations (Brummer et al. 1997; Burton 1987; Chung et al. 2003; Mao et al. 2013; Shannon et al. 1972; Wehrmann et al. 1987). Environmental influences on seed composition include temperature during seed development, light, moisture, and nutrient status of the plant (Allen et al. 2009; Dornboss and Mullen 1992; Huber et al. 2016; Iyer et al. 2008; Mourtzinis et al. 2017; Paek et al. 1997; Piper and Boote 1999; Rotundo and Westgate 2009; Vollmann et al. 2000). Genotypes may respond differently in different environments, but the genotype x environment interaction

effects do not, in general, result in drastic changes in rank among environments, so selection based on one or a few environments can be effective for seed protein and oil concentration (Cianzio et al. 1985; Zhe et al. 2010). Of greater concern is the significant negative phenotypic and genotypic correlations that exist between seed protein and seed oil concentration, and the general negative relationship observed between seed protein concentration and yield (Bandillo et al. 2015; Brim and Burton 1979; Burton 1987; Cober and Voldeng 2000; Hyten et al. 2004; Johnson et al. 1955; Kwon and Torrie 1964; Phansak et al. 2016; Vaughn et al. 2014; Wehrmann et al. 1987; Wilcox and Guodong 1997). Seed oil concentration shows a generally positive correlation with yield (Eskandari et al. 2013). As a consequence of those correlations among traits, selection for increased yield during the past 50 years has resulted in a general decline in seed protein concentration and a corresponding increase in average seed-oil concentration (Rincker et al. 2014).

Is it possible to continue to increase soybean seed yield and meet the seed composition needs for processors and end users? That is the current challenge. Increasingly, soybean breeders are focused on enhancing genetic gains for soybean yield to meet projected global demand and improve average seed protein concentration in the range required to meet processor expectations. There is a range of protein and oil concentration in the whole seed that will allow processors to meet expectations for oil yield and meal protein content (Brumm et al. 2005). For example, soybean seeds with about 33% protein on a 13% moisture basis may still be able to produce a soybean meal with the required minimum 47.5% protein content, if the seed oil concentration is high enough, around 20.5%. Likewise, a soybean seed with 37% protein on a 13% moisture basis can yield the required 48% protein meal after processing but will most likely not produce more than 4.5 kg (10 pounds) of oil. Soybean seeds with higher protein concentrations that produce a higher-protein meal are not required, because there is no premium for higher seed protein concentration above 48.5% in commodity markets. So, the targets for soybean seed composition can be based on the processed outputs expected from soybeans with a particular seed composition, so that the balance of oil, protein and carbohydrates in the seed becomes the selection target, and not a particular seed protein or oil concentration per se. Brumm and Hurburgh (1990) provide a model to estimate product outputs and determine estimated processed value of soybeans based on composition of the whole seeds.

After nearly 30 years of experiments to identify genomic regions associated with seed composition and other traits, QTLs affecting soybean seed protein, oil and carbohydrate composition have been mapped on all 20 chromosomes using both pedigree-based and association mapping approaches (Bandillo et al. 2015; Brummer et al. 1997; Diers et al. 1992; Hwang et al. 2014; Li et al. 2018; Mansur et al. 1993; Phansak et al. 2016; Vaughn et al. 2014; SoyBase.org). These results are consistent with expectations, given the complex metabolism and genetic regulatory networks involved in nitrogen fixation, uptake and assimilation, and lipid, starch, and protein synthesis and storage during seed development (Carter and Tegeder 2016; Hajduch et al. 2005; Iyer et al. 2008; Jones and Vodkin 2013; Le et al. 2010; Pelletier et al. 2017; Weber et al. 2005). Two QTLs, however, have been shown to have a signifi-

cant positive effect on seed protein concentration: one on chromosome 20 and the other on chromosome 15 (Bandillo et al. 2015; Brummer et al. 1997; Diers et al. 1992; Phansak et al. 2016; Warrington et al. 2015). First reported by Diers et al. (1992), subsequent studies confirmed significant positive additive effects on seed protein concentration for both the Chr 15 and Chr 20 QTLs, but they are associated with significant negative effects on seed oil concentration and yield, though yield reduction for the Chr 15 QTL is not significant in some environments (Brzostowski et al. 2017; Chung et al. 2003; Fasoula et al. 2004; Kim et al. 2016b; Nichols et al. 2006; Sebolt et al. 2000). In light of current knowledge and evidence, it seems unlikely that allelic variation in a single gene will be able to produce a phenotype that meets the yield and seed composition targets for seed companies, farmers, and end users.

With more than 80% of soybean meal used for feeding monogastric animals like poultry and swine, other specific targets like reduction of raffinose and stachyose in the seed may add value to the meal (Dierking and Bilyeu 2009a; Kerr and Sebastian 2000). Some mutant sources conditioning lower raffinose and stachyose in the seed, like the *myo*-inosotol 1-phosphate synthase gene, also showed reduced field emergence of seedlings and that mutation affected seed phytic acid content in addition to RFO accumulation (Hitz et al. 2002; Meis et al. 2003; Oltmans et al. 2005; Sebastian et al. 2000). Other genes in the pathway affect raffinose and stachyose accumulation in the seed but do not influence phytic acid accumulation and show no effect on seedling emergence (Dierking and Bilyeu 2008, 2009b; Obendorf and Kosina 2011; Obendorf et al. 2008, 2009; Valentine et al. 2017). Besides specific genes with known functions, other QTLs that modify sugar levels in soybean seeds have been reported, and there is significant variation in the USDA Soybean Germplasm Collection for total sugars in mature seed (Hou et al. 2009; http://soybase.org). This suggests that breeding progress can be made with appropriate selection of parents and population development to identify soybean lines with increased total protein and oil and decreased soluble carbohydrates.

Besides protein meal, oil is the other main product of soybean seed processing. The primary use for soybean oil is as a vegetable oil for human food applications, but significant use in the biodiesel industry accounts for increasing consumption of soybean oil (soystats.com). While soybean oil already is a high-quality oil for multiple uses, some modification of fatty acid composition can improve its value in both food and industrial applications. The two main targets are increased oleic acid and decreased linolenic acid content in the oil. Decreased linolenic acid reduces the total polyunsaturated fatty acids in the oil and thus improves shelf life and purportedly also suitability for frying applications, though reports differ (Mounts et al. 1994; Tompkins and Perkins 2000). Increased oleic acid in the oil, above 75%, improves oil quality for most food applications including frying, and also improves some characteristics for use in biodiesel and other industrial uses (Kinney and Knowlton 1997; Tat et al. 2007). The main pathway for fatty acid synthesis in soybean seeds is well characterized, and modification of specific genes in the elongation or desaturation steps of the pathway have been targeted by selection or biotechnology for modification of the soybean oil profile (Clemente and Cahoon 2009). A high oleic

modification developed through biotechnology has been shown to be stable over environments and have no deleterious effects on yield or other seed composition or agronomic traits (Graef et al. 2009). Soybeans with high-oleic acid developed through combining mutant Fad2 alleles, however, showed significant variation across environments for the high-oleic traits (Lee et al. 2012; Pham et al. 2010). Likewise, use of mutant alleles to achieve reduced linolenic acid concentrations in the seeds that are stable across environments requires the accumulation of recessive alleles for at least three loci, which may complicate management in a breeding program (Bilyeu et al. 2011).

Some recent studies have begun to identify key genes in networks governing seed development, which may offer some insights for targets for selection or modification (Chao et al. 2017; Liu et al. 2012a; Qi et al. 2018; Zhang and Wang 2018). Roesler et al. (2016) reported on an improved diacylglycerol acyltransferase (DGAT) variant that increased seed oil concentration in soybean seeds, with corresponding increase in protein concentration and decrease in total soluble carbohydrates in the seed; no data for yield were reported. Dobbels et al. (2017) identified a putative gene target from screening a soybean fast neutron mutant population; the KASI ortholog resulted in decreased oil concentration and increased sucrose, with no significant difference in protein concentration. However, the oil concentration in the homozygous translocation line was 8.8%, compared with 19.7% in the wild type line, and linolenic acid increased to 15.3%, compared with 7.4% in the wild type.

For complex traits like soybean seed composition and yield, a systems genetics approach may help to improve understanding and develop better strategies to modify target traits (Baliga et al. 2017; Lempe et al. 2013; Mizrachi et al. 2017; Nadeau and Dudley 2011). Better characterization of environment, including edaphic factors, weather variables, and photosynthetically active radiation can improve understanding of environmental effects and modeling of genotype-environment interactions. Field-based, high-throughput phenotyping technologies can provide a wealth of additional data on crop growth and development, photosynthesis, canopy temperature and plant stress, and other relevant traits (White et al. 2012). Data quality is paramount, with appropriate experimental design, population structure and size, data collection protocols and technologies, and analytics. Significant progress has been made in soybean breeding for yield and compositional quality, aided by the two most commonly used high-throughput phenotyping tools for yield and composition: the plot combine and NIRS for rapid estimation of soybean seed composition. Highly effective calibrations have been developed for evaluation of seed protein and oil concentration and some fatty acids, but other seed component traits that are not correlated with protein and oil cannot be effectively measured with NIRS (Esteve Agelet and Hurburgh 2014; Karn et al. 2017; Kovalchenko et al. 2006a, b). Rapid and accurate measurement of compositional quality is needed, not only to facilitate research and development, but also to assess quality specifications at the point of sale for such traits. With extensive genotype, phenotype and environment information on large and diverse sets of lines, genomic prediction models may increase efficiency and effectiveness of selection for yield and seed quality traits (Bernardo 2014; Duhnen et al. 2017; Heffner et al. 2009; Sleper and Bernardo 2018).

12.11 Opportunities for Continued Genetic Improvement

Farmers have practiced genetic improvement of domesticated soybean for thousands of years (Carter et al. 2004). In the first half of the twentieth century, farmers began outsourcing many of their activities including plant breeding and commercial seed production because seed companies were more efficient at developing improved cultivars and preparing seed for planting. For some, the term plant breeder is associated with romantic images of Luther Burbank, Nikolai Vavilov, George Sprague and, of course, Norman Borlaug. Curiously, none of these names are associated with soybean. Initially, modern plant breeders designed, developed and implemented breeding systems to translate genetic discoveries into improved cultivars as well as systems involving practical aspects of preparing foundation, registered and certified seed for large scale agronomic field trials (Fehr 1991). Like the soybean farmers who preceded them, modern soybean breeders began to specialize or collaborate with, and outsource to specialists. Today, no single individual is responsible for all aspects of genetic improvement, cultivar development, cultivar placement and seed preparation. Rather, PhD-educated soybean breeders are expected to collaborate with specialists to coordinate and manage activities that generate data to support timely decisions at appropriate stages in systems that have been inherited from prior generations of soybean breeders. While some would prefer to reserve the term *field soybean breeder* for those who participate in conducting, assessing and selecting potential cultivars based on whole plant phenotypes grown in field plots, it is essential to recognize that soybean breeding now consists of teams composed of specialists from multiple disciplines.

The modern era of soybean breeding began in 1936 (Hartwig 1973) when agronomists at agricultural experiment stations in the North Central Region of the US began to design soybean breeding systems. These first soybean breeders were cognizant of response to selection and genetic transmission to subsequent generations based on the theoretical relationship $\Delta G_c = Sh^2$ (Fisher 1930), a variation of the genetic gain equation described earlier in this chapter. ΔG_c refers to genetic gain due to genetic inheritance in cycle c, where S is the selection differential between the average phenotypic values of a selected proportion of a population and the overall average of the population, i.e., $\overline{X}_s - \overline{X}_p$, and h^2 refers to heritability, i.e., the proportion of total phenotypic variability inherited by progeny from crosses among selected individuals in cycle c. Equivalent forms of ΔG include $Sh^2 = \iota\sigma_p h^2 = \iota\sigma_A h$.

Modern soybean breeders recognized that the easiest way to increase ΔG_c was to reduce the number of years required for each breeding cycle (Fehr 1991). Initial designs of soybean breeding systems were constrained primarily by the organism's growth, development and reproductive biology. Development of *winter nurseries* in tropical locations enabled soybean breeders to obtain seed while increasing the magnitude of additive genetic variability through advanced self-pollinated generations, e.g., F_2 to F_3, F_3 to F_4, etc. In the first decade of the twenty-first century, commercial soybean organizations further reduced cycle time through development of continuous nurseries in tropical and high latitude locations in both northern and

southern hemispheres. Continuous nurseries were enabled by emergence of information technologies and logistical software systems that track and deliver seed, as well as financial support from seed sales. Continuous nurseries provided the ability to cross and develop replicable progeny throughout the year. Even the number of years dedicated to field trials were reduced through use of locations at equivalent latitudes in opposite hemispheres. Consequently, time required per cycle was reduced from more than a dozen years in the 1960s to as little as 5 years (2 years to develop replicable lines and 3 years of field trials) today.

While innovations in soybean breeding have been driven by dividing ΔG_c by the number of years required to complete a cycle and breeding costs based on financial metrics, such metrics create a counter intuitive outcome: They are maximized when there is no investment or time devoted to genetic improvement. Rather than attempting to minimize investment costs and time, it seems more appropriate to maximize benefits, i.e. return on investment, for making breeding decisions (Cameron et al. 2017). More expensive, shorter times to complete a project can bring greater benefits by being first to market or increasing market share and thus justify greater investments of time and money.

With the emergence of *omics* technologies, many individuals interested in soybean genetic improvement became soybean molecular geneticists, i.e. specialists in genetic discovery. Using both forward and reverse genetic approaches discovery rates of desirable alleles in germplasm resources and gene banks associated with and responsible for desirable traits has rapidly grown (Blumel et al. 2015; Kumar et al. 2010; Leung et al. 2015). These discoveries have been catalogued in publicly-supported databases. Soybean breeders have two excellent Bioinformatics resources, Soybase (soybase.org) and LIS (legumeinfo.org), to help them identify candidate genes for many important agronomic traits including seed oil composition, tolerance to iron deficiency chlorosis, resistance to soybean cyst nematode, sudden death syndrome and brown stem rot. However, commitments by soybean molecular geneticists are needed to catalogue their discoveries, because both LIS and Soybase have very little budget for curation. Recently, Yu et al. (2016b) advocated *turbocharging* the discovery process through application of genotyping-by-sequencing (GBS), genomic estimated breeding values (GEBVs) and empirical validation. If an accelerated discovery system is implemented in soybean, there will be a serious bottleneck in transmitting discoveries by soybean molecular geneticists to breeding teams.

None-the-less, in the short term, soybean breeders can design breeding schemes to stack or pyramid desirable alleles while increasing yields in improved cultivars. Consider, for example, that vegetable oil extracted from commodity soybean cultivars have high concentrations of polyunsaturated fatty acids (PUFA) including 54% linoleic and 8% linolenic acid (Wilson 2004). These fatty acids are responsible for rancidity and shortened storage time (Warner and Fehr 2008). Food processors hydrogenate soybean oil to reduce the PUFA, however this results in production of *trans* fatty acids that contribute to obesity and heart disease. As discussed previously, commercial seed companies have used transgenic approaches to reduce the concentration of PUFA and increase the concentration of oleic acid in soybean seed

(Fillatti et al. 2013; Knowlton 1999). Alternatively, scientists at USDA-ARS have identified naturally occurring alleles at 2 FAD2 loci that increase oleic acid concentrations and 3 FAD3 loci that lower linolenic acid concentrations (Anh-Tung et al. 2011; Bilyeu et al. 2003, 2005, 2006, 2011; Chappell and Bilyeu 2007; Dierking and Bilyeu 2009a; Kim et al. 2015; Pham et al. 2012; Thapa et al. 2016). The optimal combination of alleles for purposes of meeting both processing and flavor demands appears to be one with a favorable allele at a FAD2 locus and 3 desirable alleles at all 3 FAD3 loci (Pham et al. 2012). To date, there is no experimental evidence that oil composition is negatively associated with yield (Graef et al. 2009; Warner and Fehr 2008). To further evaluate possible associations of oil composition with yield, public soybean breeders are using a backcrossing strategy to introgress 4 desirable alleles into elite lines that are adapted to several maturity zones (plantsci.missouri.edu/faculty/bilyeu.cfm).

Is intercrossing to stack all desirable alleles into a single line followed by backcrossing the best breeding strategy to accomplish the task? Would genome editing be a more efficient and effective approach? The answer will depend, in part, on whether the breeding team has legal negotiated access to genome editing methodology and sufficient resources to address regulatory requirements. More importantly, what metrics are needed to address such questions?

Unfortunately, the quantitative impact of new discoveries and/or technologies on any specific breeding program cannot be answered using the algebraic forms of ΔG or its equivalent multi-trait index (Hazel 1943; Smith 1936), $\Delta H_c = \imath r_{IH} \sigma_H = \sum a_i \Delta G_{c_i}$.

Because selection theory makes simplifying assumptions about the genetic architecture (infinitesimal additive model), genomic organization (no linkage), population structure (Hardy-Weinberg equilibrium) and the nature of selection (single stage truncation). Because none of these assumptions are correct for any plant breeding program, computer simulations using reasonable models for genetic architectures and inheritance (Cooper and Podlich 2002; Fraser and Burnell 1970; Peccoud et al. 2004; Tinker and Mather 1993) were advocated as an approach to explore alternative breeding designs (Cress 1967; Li et al. 2012; Podlich and Cooper 1998; St Martin and Skavaril 1984; Sun et al. 2011).

With increasing computing power, many plant scientists with interests in statistical methods became specialists in quantitative genetics. In the last 20 years, quantitative geneticists have used simulations as their primary approach to investigate a large number of questions associated with impacts of discoveries and technologies on genetic gain. Initially, many of these studies focused on impacts due to non-additive genetic architectures dependent on environmental signals (Cooper and Podlich 2002; Cooper et al. 2002; Peccoud et al. 2004) as well as how to best allocate resources in terms of numbers of plots and environments (Longin et al. 2007; Wang et al. 2003, 2004). There have been many publications on the challenges of marker assisted backcross breeding strategies to introgress single gene traits. See the introduction in Cameron et al. (2017) for a sample of this large literature. As the numbers of gene discoveries increased, simulations have been used to address the question of how to best pyramid or stack these genes into a single background (De

Beukelaer et al. 2015; Servin et al. 2004; Wang et al. 2007; Xu et al. 2011). Perhaps the largest number of publications that have used simulation approaches were devoted to the impact of genomic selection on genetic gain. See the introduction in Gaynor et al. (2017) for a sample of this literature.

The power of simulations to investigate possible breeding strategies prompted several large commercial seed companies to invest in teams consisting of quantitative geneticists, computer scientists and software developers for purposes of developing predictive models (Bernardo 1996; Henderson 1985) and simulation software. Note that prediction is an emerging discipline that is fundamentally changing how science questions are being approached: from observation-generated hypotheses to model-generated hypotheses (Johnson 2007; Peccoud et al. 2004). In the public sector, some quantitative geneticists have worked with software developers to create simulation software packages including AlphaSim, COGENFITO, GENEFLOW, GREGOR, MPB, PLABSIM, QUGENE/QuLine and *selectiongain*. Despite these efforts there are no comprehensive, open-source software packages, although there have been efforts to standardize simulation criteria for purposes of replicating simulation studies (Daetwyler et al. 2013). It is not clear if the concept of a core facility to help public soybean breeders make breeding design decisions would be funded.

The application of simulations to investigate how to best modify existing breeding projects raises a question about the underlying premise. Sixty years ago, if we had known the organization of the soybean genome, genetic networks and architectures responsible for agronomic traits, and had had access to tools of digitized phenomics, genomic prediction, genome editing and predictive crop modeling, would our breeding designs be completely different from what we have inherited and are attempting to modify? The obvious corollary is, should we now consider redesigning soybean breeding systems to be optimal?

In 2009, researchers at Syngenta, US (Byrum et al. 2016) asked whether their soybean breeding program could become more efficient by first recognizing that it consisted of subsystems for genetic improvement, trait introgression, cultivar development and cultivar placement. Next, they disentangled the sub-systems and identified about 250 decision points in the cultivar development subsystem. If these decisions were independent and binary, at least 2^{250} possible designs could be considered to optimize cultivar development. Based on development of novel metrics for determining realized genetic gains (Byrum et al. 2017) and simulations, they redesigned and implemented new cultivar development systems resulting in over USD 287M cost savings, greater realized genetic gains and the Edelman prize in 2015 (informs.org/About-INFORMS/News-Room/Press-Releases/Syngenta-Wins-2015-INFORMS-Edelman-Prize).

In common language, the term optimal is applied to any attempt to make a system better, whereas Merriam-Webster considers optimization to be the process of designing systems to be as effective and efficient as possible through mathematical models and procedures (merriam-webster.com/dictionary/optimization). Operations research (OR) is a discipline in applied mathematics devoted to the study of optimization. OR emerged out of the need for analytics to support dynamic decision-making with uncertain outcomes during the WWII. Some of the first civilian

applications of OR were in agriculture (Boles 1955; Heady 1954; Heady and Pesek 1954; Rendel and Robertson 1950; Robertson 1957). With one exception (Johnson et al. 1988), OR approaches to designing plant-breeding systems were largely ignored until about 10 years ago (Akdemir and Sanchez 2016; Cameron et al. 2017; Canzar and El-Kebir 2011; De Beukelaer et al. 2015; Han et al. 2017; Xu et al. 2011).

There are three components to optimization models: objective functions, decision variables and constraints. Objective functions represent metrics that need to be minimized or maximized, e.g. minimize costs, maximize the probability of success, minimize time to market, etc. Decision variables are components of the objective functions that we can control, e.g. how many plants to grow per generation, how many markers to assay and how many plants to select each generation. Constraints are limitations on the decision variables and objective functions, e.g. budget restrictions, biological growth, development and reproduction, and size of field plots.

As previously noted, the OR professional society has become aware of the need for redesigning efficient plant breeding systems (Byrum et al. 2016, 2017). Of particular interest for applied mathematicians is the challenge of bi-level optimization, i.e. the need for novel algorithms to design optimal programs composed of multiple integrated systems. For example, it makes little sense to have an optimal cultivar development system if such as system minimizes the effectiveness of an associated genetic improvement system.

Ultimately, questions about trade-offs and optimization depend on measurable objectives framed in terms of returns on investment. Genetic gain considered in terms of forecast benefits will enable quantification of the trade-offs between time and cost. Unfortunately, soybean breeders no longer spend much time with soybean producers and thus have little knowledge of how to forecast benefits. Forecasting benefits, like predicting genetic outcomes, is not simply a matter of conducting market surveys. Rather, it should be recognized that forecast benefits are due to uncertain outcomes from stochastic processes, similar to transmission genetics. Systems engineers have been successfully integrating forecasts and risk assessments for optimal outcomes from projects that involve uncertainty since the inception of OP (Birge and Louveaux 2011). We suggest that it is time for plant breeders to return to their original role as designers of plant breeding programs by learning the principles of OR and collaboration with applied mathematicians.

12.12 Conclusions and Prospects

Soybeans are an economically important global commodity crop and prime source of high quality protein and oil for livestock, aquaculture, industrial uses and human consumption. Agronomic improvements and both public and private breeding and basic research efforts continue to advance yields, genetic gain, stress resistance, quality and output traits.

Increasing soybean yield continues to be the most important selection criteria for soybean breeders and the primary factor for profitability for soybean producers. Marker-assisted selection has been highly effective in the introgression of qualitative traits, but not for complex traits like yield. Genomic selection can improve breeding for yield but decreasing the cost of markers and developing effective predictions of genetic gain are required. Breeders also must take advantage of opportunities for utilizing diverse germplasm for important traits. There is less than 1% of the available US germplasm that has been appropriated to create current cultivars. Much is yet to be learned about the genetics of seed yield and how breeders can better select for useful genetic diversity within the wide range of germplasm that is available. There is evidence that unique genetic diversity for yield exists in exotic soybean germplasm and some successes have been reported for introgressing genes from wild soybean and *Glycine tomentella* that can increase the yield of at least some commercial cultivars. When priorities are set for allocating more resources, these results deserve more attention than they are currently receiving from US soybean breeding programs.

Soybeans are negatively impacted by many insect pests, pathogens, weeds and abiotic stressors so yield preservation also must be a priority for basic research, breeding and cultivar development. Agronomic and cropping systems improvement, combined with efforts to develop new cultivars with durable tolerance or resistance to various abiotic and biotic stressors represent the most integrated and effective ways to combat soybean yield losses. Here we have emphasized work addressing tolerance to environmental and abiotic stressors because expanding soybean plantings and changing climate have made these stressors more prominent in US soybean production. A combination of traditional breeding, marker-assisted selection and transgenic approaches can make breeding for pest resistance and stress tolerance more successful. Although promising advances have been made in various areas of research on resistance to abiotic and biotic stressors, challenges remain in understanding the underlying mechanisms of plant-pathogen, plant-insect and plant-abiotic stressor interactions, and combining multiple resistance and tolerance traits into commercial cultivars.

Development of food-grade cultivars requires the selection of multiple quantitative traits. Commonly, breeders apply independent culling levels to each trait. Due to the correlation of key traits (protein, yield, sugar), multi-trait selection indices may be a more apt method (Hazel 1943; Smith 1936; Williams 1962). However, the application of multi-trait selection indices will require working in collaboration with soybean processors to determine the specific economic weights for each trait. Multi-trait indices can also effectively be applied in combination with genomic selection (Ceron-Rojas et al. 2015; Heffner et al. 2011). An improved understanding of how seed composition trait values impact the final soyfood product for individual processing methods would facilitate selection of multiple traits, regardless of whether multi-trait indices or culling levels are applied.

When considering all aspects of soybean production and soybean uses, soybean improvement has both benefited from and served as a platform for genetic and genomic technologies discovery, development and deployment for improving com-

mercial cultivars. The development of breeder-friendly molecular markers was initially a key enabler that redefined how soybeans were bred. The markers enable numerous genes to be mapped and selected during the breeding process. This not only increased selection accuracy but also increased genetic variance by decreasing the cost and time of screening many phenotype traits. The public effort to sequence the soybean genome, along with a revolution in sequencing technology, enabled genomics to be further deployed into the breeding process. The development and utilization of current genomics technology has shifted the bottleneck from collecting genomic data to collecting phenotypic data. This stimulated the current discovery efforts by the public sector to find better, faster and less expensive methods for collecting phenotypes. The convergence of genomics and phenomics will drive great advances in soybean breeding.

The approaches and examples presented in this chapter suggest that induced mutagenesis is a useful tool in soybean for introducing heritable genetic variation that leads to new cultivar development and gene discovery. Therefore, mutagenesis is a critical tool for developing novel variation that can be used by breeders in cultivar development or give insights into the functions of genes that may be screened for natural variation or modified in the future for specific agronomic or crop improvement purposes.

The available molecular breeding techniques and tools of soybean biotechnology are equal to those available for crops like rice and corn, and far superior to those available for the other crops. Nevertheless, while the DNA-based aspects of the technology have advanced greatly, the cell culture methodology that accompanies DNA technology has advanced very little over the past 20 years. Regeneration is still done using organogenesis from cotyledonary nodes or leaf axes, or by somatic embryos obtained from immature seeds. Transformation depends on *Agrobacterium* for organogenesis, or biolistics for somatic embryogenesis (Widholm et al. 2010). Very few cultivars are amenable to biotechnology applications, which are still lengthy, laborious and relatively inefficient in soybean. Thus, the development of large numbers of engineered plants remains out of reach for the public sector. The recent availability of the Wisconsin Crop Innovation Center (formerly, the Monsanto Agracetus soybean transformation facility) as of 2017 may make higher throughput possible. At the least, it should make more cultivars amenable to transformation.

The last, and perhaps greatest, barrier to biotechnology applications is regulatory, which in turn influences public acceptance. Regulatory requirements around the world have long exceeded the size of any risk that may be present (Conko et al. 2016). The cost of regulatory approval has therefore become cost-prohibitive for the majority of engineered traits, beyond currently approved herbicide tolerance traits, in soybean, which in turn, means that new engineered traits may never see the marketplace.

The expense and improbability of an engineered trait ever reaching the market has been a strong disincentive to invest further in the technology. At the same time, these have been powerful incentives to focus on genome editing. Genome editing is the latest biotechnology advancement with broad application potential for accelerating crop improvement targets including yield, performance and quality. Genome

edits include precise modifications, such as small genetic insertions and deletions, as well as small or large gene modifications and replacements. The most common techniques in this new class of genome editing tools include: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9). All of these rely on special nucleases that cut one or both strands of a double-stranded DNA molecule at predetermined locations and allow subsequent modifications. CRISPR, in particular, seems to have great potential as a genome editing tool for soybean, given the increasing body of knowledge related to the soybean genome sequence and function, and the relative ease and efficiency of CRISPR systems. There are some technical challenges that must be overcome for the soybean CRISPR system, but rapid advances are being made. Yet, while genome editing is a powerful technology, it may not replace engineering for many traits. Furthermore, the regulatory status of genome editing remains unclear (Parrott 2018). In the US, the USDA is taking a logical approach in not regulating most edited traits. The FDA and EPA positions remain unclear, as does the stance of the European Union (EU) and China. The Court of Justice of the European Union recently decided that organisms obtained by genome editing mutagenesis are GMOs (genetically modified organisms) and are, in principle, subject to the obligations laid down by the GMO Directive. This decision, if it stands, presents a major obstacle to genome editing for soybean and other plant species improvement. Many other groups around the world are actively lobbying to get genome edited plants to be regulated like engineered traits. If governments determine that edited plants should be regulated like transgenic plants, the technology may not live up to its potential, as once again, the cost of regulation will exceed the value of many traits. Finally, possible new labeling requirements in the US may greatly add to the cost of genome edited and other biotechnology crops and food plants. As regulatory policy is formulated and refined, it will be critical for scientists, breeders, and seedsmen to provide scientifically valid evidence to regulators and help educate the public on the true nature and potential of biotechnology. In spite of these current technical challenges and regulatory and acceptance hurdles, the potential for genome editing for soybean improvement is great enough to maintain this as a viable technology for further development and application.

With multiple competing objectives there may not be a single maximum or minimum solution for addressing future soybean breeding and cultivar development needs and opportunities. Instead, there will be an optimal set of solutions that fall on what is referred to as the Pareto-frontier where it is not possible to improve one objective without degrading at least one of the other objectives. For example, there is a tradeoff between a budget and the probability of achieving the genetic goals under different project deadlines. Plots of Pareto frontiers provide a clear and concise representation of the tradeoffs among the objectives and enable decision makers to quantify the trade-offs among clearly defined objectives. Pareto optimality also enables funders and investors to decide whether limited efforts have any chance of success.

In the future, it seems likely that executive managers and funding agencies will expect plant breeding teams to provide analyses of designed projects using approaches from operations research (OR). Indeed, with the recent mergers of large crop protection and large seed companies there is evidence that future plant breeding teams will require knowledge of OR to design, develop and implement systems that will optimally address multiple objectives. In other words, the agricultural art of plant breeding is becoming a highly integrated and more engineering-like discipline.

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Appendix I: Research Institutes in the US Relevant to Soybean

Institute	Areas of specialization	Website
Auburn University	Breeding, Agronomy, Basic Research	auburn.edu
BASF Company	Seed and Crop Protection	basf.com
Bayer Company	Seed and Crop Protection	cropscience.bayer.us
Clemson University	Breeding, Agronomy, Basic Research	clemson.edu
Cornell University	Breeding, Agronomy, Basic Research	cornell.edu
Corteva Agriscience	Seed and/or Crop Protection	corteva.com
FMC Corporation	Crop Protection	fmccrop.com
Iowa State University	Breeding, Agronomy, Basic Research	iastate.edu
Kansas State University	Breeding, Agronomy, Basic Research	k-state.edu
Louisiana State University	Breeding, Agronomy, Basic Research	lsu.edu
Michigan State University	Breeding, Agronomy, Basic Research	msu.edu
Mississippi State University	Breeding, Agronomy, Basic Research	msstate.edu

(continued)

Institute	Areas of specialization	Website
North Carolina State University	Breeding, Agronomy, Basic Research	ncsu.edu
North Dakota State University	Breeding, Agronomy, Basic Research	ndsu.edu
Oklahoma State University	Breeding, Agronomy, Basic Research	go.okstate.edu
Pennsylvania State University	Breeding, Agronomy, Basic Research	psu.edu
Purdue University	Breeding, Agronomy, Basic Research	purdue.edu
Rutgers University	Breeding, Agronomy, Basic Research	rutgers.edu
South Dakota State University	Breeding, Agronomy, Basic Research	sdstate.edu
Syngenta Company	Seed and Crop Protection	syngenta.com
Texas A&M University	Breeding, Agronomy, Basic Research	tamu.edu
The Ohio State University	Breeding, Agronomy, Basic Research	osu.edu
University of Arkansas	Breeding, Agronomy, Basic Research	uark.edu
University of Delaware	Breeding, Agronomy, Basic Research	udel.edu
University of Georgia	Breeding, Agronomy, Basic Research	uga.edu
University of Illinois	Breeding, Agronomy, Basic Research	illinois.edu
University of Kentucky	Breeding, Agronomy, Basic Research	uky.edu
University of Maryland	Breeding, Agronomy, Basic Research	umd.edu
University of Minnesota	Breeding, Agronomy, Basic Research	umn.edu
University of Missouri	Breeding, Agronomy, Basic Research	missouri.edu
University of Nebraska	Breeding, Agronomy, Basic Research	unl.edu
University of Tennessee	Breeding, Agronomy, Basic Research	utk.edu
University of Wisconsin	Breeding, Agronomy, Basic Research	wisc.edu
Virginia Tech University	Breeding, Agronomy, Basic Research	vt.edu

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